CANCER RESEARCH

VOL. 9 OCTOBER 1949 No. 10

CONTENTS

THE OFFICIAL ORGAN OF THE

AMERICAN ASSOCIATION FOR CANCER RESEARCH, INC.

Published by THE UNIVERSITY OF CHICAGO PRESS

CANCER RESEARCH

This journal is sponsored by The American Association for Cancer Research, Inc.; The Anna Fuller Fund; Cancer Research Division, Donner Foundation, Inc.; The Jane Coffin Childs Memorial Fund for Medical Research; and The Elsa U. Pardee Foundation.

CANCER RESEARCH is published monthly for Cancer Research, Inc., by the University of Chicago Press, 5750 Ellis Avenue, Chicago 37, Illinois. The subscription price is $7.00 per year; the price of single copies is $1.00. Orders for less than a full year will be charged at the single copy rate. Postage is prepaid by the publishers on all orders from the United States and its possessions, Argentina, Bolivia, Brazil, Chile, Colombia, Costa Rica, Cuba, Dominican Republic, Ecuador, Guatemala, Haiti, Republic of Honduras, Mexico, Morocco (Spanish Zone), Nicaragua, Panama, Paraguay, Peru, Rio de Oro, El Salvador, Spain (including Balearic Islands, Canary Islands, and the Spanish Offices in Northern Africa; Andorra), Spanish Guinea, Uruguay, and Venezuela. Postage is charged extra as follows: For Canada and Newfoundland, 48 cents on annual subscriptions (total $7.48), on single copies 4 cents (total $1.04); for all other countries in the Postal Union, $1.20 on annual subscriptions (total $8.20), on single copies 10 cents (total $1.10).

The following is an authorized agent:

For the British Empire, except North America and Australasia:

Cambridge University Press, Bentley House, 200 Euston Road, London, N.W. 1, England. Prices of yearly subscriptions and of single copies may be had on application.

Business communications, remittances (in United States currency or its equivalent), and subscriptions should be addressed to THE UNIVERSITY OF CHICAGO PRESS, 5750 Ellis Avenue, Chicago 37, Illinois. All other communications should be addressed to Paul E. Steiner, M.D., University of Chicago, Chicago 37, Illinois.

Claims for missing numbers should be made within the month following the regular month of publication. The publishers expect to supply missing numbers free only when losses have been sustained in transit and when the reserve stock will permit.

No responsibility is accepted by the Editors, by Cancer Research, Inc., or by the publishers of *CANCER RESEARCH* for opinions expressed by contributors.

Entered as second-class matter, February 15, 1949, at the post office at Chicago, Ill., under the Act of March 3, 1879.

CANCER RESEARCH

VOLUME 9 OCTOBER 1949 NUMBER 10

Diet and Azo Dye Tumors: Effect of Diet During a Period When the Dye is Not Fed*

C. C. CLAYTON,† PH.D., AND C. A. BAUMANN, PH.D.

(From the Department of Biochemistry, College of Agriculture, University of Wisconsin, Madison 6, Wisconsin)

Although it is well recognized that rats fed azo dyes develop tumors at rates that depend upon the diet fed with the dye (28), the mechanism by which this is accomplished is unknown. One possibility is that pertinent diets alter the metabolism of the dye and thus alter the concentration of effective carcinogen; another, that diet can effect changes in normal cellular constituents that either enhance or minimize the sensitivity of the tissue to the carcinogen. Still another possibility is that appropriate diets might modify those phases of the carcinogenic process that continue after the carcinogen is no longer present in the tissues. Dietary effects in the latter categories would have a wider significance in the cancer problem than those in the first. Accordingly hepatic tumors were induced in rats by the feeding of a synthetic ration containing the potent azo dye, *m'*-methyl-*p*-dimethylaminoazobenzene (*m'*DAB), interrupted by the feeding of experimental diets containing no dye. In a few parallel studies the riboflavin contents of the livers and the coagulability of liver homogenates were also measured.

METHODS

Comparable groups of 12 to 15 young adult rats (usually males) weighing approximately 200 grams were placed in wire bottom cages and given food and water *ad libitum*. The consumption of food was measured at intervals on all groups. In experiments involving caloric restriction the rats were kept in individual cages and the desired amount of food was fed daily. The basal diet was that used in previous studies (8, 12, 28): extracted casein 12, salts 4, corn oil 5, and glucose monohydrate (Cerelose) to 100 with vitamins added at the following levels in mg./kg. of diet: thiamine 3, pyridoxine 2.5, calcium pantothenate 7.5, riboflavin 2, and choline 30. In the final dietary series (Table 2, groups 29 to 33) and in the analytical series (Table 4) all diets contained 1000 mg. of choline per kg. Each rat also received 2 drops of halibut liver oil every 4 weeks.

For the incorporation of *m'*-methyl-*p*-dimethylaminoazobenzene (*m'*DAB) into the rations, the dry ingredients including the vitamins were mixed first and an ether solution of the dye was evaporated onto the dry mixture. The corn oil was then incorporated into the diet and the mixture passed through a 10-mesh sieve. The concentration of dye in the ration and the periods of time the dye was fed are described below.

EXPERIMENTAL AND RESULTS

Tumor incidence after interrupted feeding of azo dyes.—In the initial experiment *m'*-methyl-*p*-dimethylaminoazobenzene (*m'*DAB) was fed at a level of 0.048 per cent to 3 groups of rats for a total of 10 weeks. The control groups received the dye continuously. Two other groups received the dye for 6 weeks followed by "periods of interruption" of 4 or 10 weeks during which the basal diet free of the dye was fed. Thereafter the carcinogen was fed for 4 more weeks, after which the dye-free diet was fed, and the experiment was terminated at the end of 29 weeks. Thus the 3 groups received the basal diet for 19, 15, and 9 weeks respectively after the last exposure to the carcinogen.

* Published with the approval of the director of the Wisconsin Agricultural Experiment Station. Supported in part by the Jonathan Bowman Cancer Fund and by a grant from the Committee on Growth, American Cancer Society.

† United States Public Health Service Predoctorate Research Fellow.

Liver tumors developed in all groups, although the interruption of the carcinogenic treatment reduced tumor incidence somewhat. In the group receiving the dye continuously, 85 per cent of the rats developed tumors; when the feeding of the dye was interrupted for 4 or 10 weeks, the incidences of tumors were 58 per cent and 61 per cent respectively (Table 1, groups 1 to 3). The results were similar in 2 other series in which 0.064 per cent of *m'*-DAB was fed for 8 weeks with "periods of interruption" ranging from 2 to 12 weeks after an initial exposure to the dye of 4 weeks. Groups receiving the carcinogen for only 4 weeks failed to develop tumors (Table 1, groups 4 and 8). The incidences of tumors were 87 and 100 per cent in the groups receiving the dye continuously (Table 1, groups 5 and 9), while in groups in which the administration of the carcinogen was interrupted, the percentages of tumors ranged from 33 to 90 per cent (Table 1, groups 6, 7 and 10 to 13). In general the number of tumors decreased as the period of interruption was extended. However, when the period of interruption was 4 or 8 weeks, tumor incidences were sufficiently high to warrant the use of this procedure in nutritional studies.

TABLE 1

LIVER TUMORS PRODUCED AFTER INTERRUPTED FEEDING OF *m'*METHYL *p*-DIMETHYLAMINOAZOBENZENE

Groups 1–3 0.048% *m'*DAB 6 wks.; Basal Diet x wks.; 0.048% *m'*DAB 4 wks.; Basal to 29 wks.
Groups 4–13 0.064% *m'*DAB 4 wks.; Basal Diet x wks.; 0.064% *m'*DAB 4 wks.; Basal to 29 wks.

Group	Weeks on basal (x wks.)	Av. init. wt. gm.	Av. wt. end 1st dye feeding gm.	Av. wt. start 2d dye feeding gm.	Av. wt. end of dye feeding gm.	Av. food consumption on dye gm./day	Survival at end of dye feeding	No. of tumors 29 wks.	Neg. survivors 29 wks.	Cirrhosis at 29 wks.	Per cent Tumors
1	0	160			183	9.8	13/15	11	1	0	85
2	4	157	182	243	220	10.2	12/15	7	4	0	58
3	10	155	184	249	236	9.3	13/15	8	3	0	61
4*	0	157	139			7.7	15/16	0	12	0	0
5	0	177			167	9.0	15/17	13	1	0	87
6	4	170	150	214	197	9.2	15/17	7	4	4	47
7	8	172	155	232	212	9.3	15/18	5	4	6	33
8*	0	194	176				6/6	0	5	1	0
9	0	202			197		11/12	11	0	0	100
10	2	184	162	209	198		11/15	9	2	0	82
11	4	181	162	231	221		10/15	9	0	1	90
12	8	191	168	272	247		13/15	7	5	0	54
13	12	189	171	300	276		9/15	6	1	2	67

*m'*DAB = *m'* methyl *p*-dimethylaminoazobenzene.

* Groups 4 and 8 received the dye for only 4 weeks.

EFFECT OF DIET DURING THE PERIOD OF INTERRUPTION

In a typical experiment *m'*DAB was fed at a level of 0.064 per cent for 4 weeks followed by 4 weeks during which various dye-free diets were fed; the basal diet containing the *m'*DAB was then fed for 4 more weeks after which the dye-free basal diet was fed for 8 weeks when the animals were killed for examination. The group fed the basal diet during the period of interruption served as a control.

High casein and riboflavin.—The levels fed during the period of interruption were 24 per cent of casein and 20 mg. of riboflavin per kg. of diet. In the first series there was a definite decrease in tumor incidence in the group fed the more adequate diet: hepatic tumors developed in 47 per cent of the group receiving the basal diet during the intermediate period as compared to 20 per cent of the animals receiving supplements of casein and riboflavin (Table 2, groups 14 and 18). A second series indicated a smaller difference between the percentages of tumor incidences, 90 *vs.* 77 per cent (Table 2, groups 23 and 24). In this series the survival of the rats in the control group was poor and the number of animals that developed tumors in the group receiving the added casein and riboflavin was actually greater than that in the control group. In a third series the incidence of tumors was essentially the same in the group fed extra casein and riboflavin during the period of interruption as in the control group (62 *vs.* 69 per cent; Table 2, groups 29 and 32). Thus it is evident that riboflavin was much less anticarcinogenic in the present experiments than in previous studies in which it was fed simultaneously with *p*-dimethylaminoazobenzene (15, 23), *p*-monomethylaminoazobenzene (20), *o'*-methyl-*p*-dimethylaminoazobenzene (8), or *m'*-methyl-*p*-dimethylaminoazobenzene (8). It is possible, therefore, that much of the effect of the vitamin is concerned with the detoxification of the azo dye or with resisting its immediate effects.

Effect of choline, methionine, and nicotinamide.—Group 26, Table 2, was fed 0.1 per cent of choline during the period of interruption. This is not a particularly high level of the vitamin in terms of current nutritional practice, but it is significantly

higher than the 0.003 per cent in the control diet. To two other groups 0.72 per cent of methionine was fed in the diet; this is the amount of methionine in a diet containing 24 per cent of casein (33). Still other groups received 0.29 per cent of nicotinamide added to the basal diet from which all choline was removed; this amount of nicotinamide could theoretically combine with all of the methyl groups in the 12 per cent of casein in the diet.

but the number of rats with tumors was actually greater in the methionine fed group than in the controls, due to the relatively poor survival of the latter (Table 2, groups 25 and 27). Thus attempts to minimize cirrhosis during the period of interruption by feeding choline and methionine had little effect on the final incidence of liver tumors, possibly because the degree of recovery after 4 weeks was already maximal in the control animals.

TABLE 2

LIVER TUMORS DUE TO *m'*DAB: EFFECT OF DIET DURING THE PERIOD OF INTERRUPTION

Groups 14–18, 23–33 0.064% *m'*DAB 4 wks.; Dye-free diets 4 wks.; 0.064% *m'*DAB 4 wks.; basal 8 wks.
Groups 19–22 0.064% *m'*DAB 4 wks.; Dye-free diets 4 wks.; 0.048% *m'*DAB 4 wks.; basal 8 wks.

Group	Diet during period of interruption	Av. wts. 0 wks. gm.	Av. wts. 4 wks. gm.	Food intake on dye-free diet gm./rat/day	Av. wts. 8 wks. gm.	Av. wts. 12 wks. gm.	Food intake on dye gm./rat/day	Survival at 12 wks.	Tumors at 20 wks.	Neg. survivors 20 wks.	Cirrh. at 20 wks.	Per cent tumors
14	Basal	170	150	13.6	214	197	9.2	15/17	7	4	4	47
15	0.29% nicotinamide	166	150	12.3	188	177	8.2	14/18	9	1	3	64
16*	" "	167	146				8.2	17/17	0	11	0	0
17	0.72% methionine	163	145	11.4	208	198	9.7	15/15	8	5	2	53
18	24% casein 20 mg. ribof./kg.	161	146	12.1	223	205	8.9	15/15	3	8	2	20
19	Basal	228	186	13.9	245	229	9.9	10/15	4	2	2	40
20	Basal restr. (63%)	220	171	8.8	183	210	10.2	9/15	6	1	2	67
21	0.29% nicotinamide	228	181	11.3	222	234	11.8	11/15	10	0	1	91
22	5 ppm. selenium	223	169	10.8	198	214	11.6	9/15	2	3	3	22
23	Basal	181	162		231	221		10/15	9	0	1	90
24	24% casein 20 mg. ribof./kg.	198	171		276	247		13/15	10	3	0	77
25	Basal	195	165	11.7	211	198	10.3	9/14	7	2		78
26	0.1% Choline	209	176	15.7	226	218	10.9	12/15	9	3		75
27	0.72% methionine	204	169	15.8	231	209		12/15	8	4		67
28	0.29% nicotinamide	201	175	13.0	220	208		9/15	6	2		67
29	Basal	193	189	14.5	256	244	12.2	13/15	8	4	1	62
30	Basal restr. (63%)	197	178	9.1	192	207	10.7	14/15	13	0	1	93
31	5 ppm. selenium	201	184	11.3	226	230	12.0	13/15	4	6	3	31
32	24% casein 20 mg ribof./kg.	200	200	15.7	308	279	13.7	13/15	9	3	1	69
33	20% corn oil	194	193	14.3	256	242	13.0	15/15	15	0	0	100

* Group 16 received dye for only 4 weeks.

The increased level of choline in the diet during the intermediate period had no effect on the final tumor incidence: tumors developed in 75 per cent of the animals receiving the higher level of choline as compared to 78 per cent incidence in the group receiving the basal diet (Table 2, groups 25 and 26). Methionine likewise appeared to be without effect on the final tumor incidence when fed during the period of interruption. In the first series the incidence of tumors was 47 per cent in the control group as compared to 53 per cent when methionine was fed (Table 2, groups 14 and 17). In a second series the percentage of tumors in the group receiving methionine was less than in that receiving the basal diet during the period of interruption,

On the other hand the incidence of tumors appeared to be increased when the diet fed during the intermediate period was made low in available methyl groups by the addition of nicotinamide. In one series the incidence was increased from 47 per cent in the group receiving the basal diet to 64 per cent in the group that had received nicotinamide (Table 2, groups 14 and 15). In another series 91 per cent of the animals on the low methyl diet developed tumors as compared to only 40 per cent of the control animals (Table 2, groups 19 and 21). In a third series the survival of the animals was poor, and only 8 of 15 animals in the nicotinamide supplemented group were alive at the termination of the experiment. In this series no increased num-

ber of tumors was seen in the group fed nicotinamide (Table 2, groups 25 and 28). The results of the three series, however, suggest that a dietary regime which prolongs cirrhosis during the period of interruption, thereby tends to increase the final incidence of hepatic tumors due to *m'*DAB. It is of interest in this connection that neither choline (20, 39), methionine (12, 39), nor nicotinamide (20) affected the incidence of liver tumors when these substances were fed with the carcinogen *p*-dimethylaminoazobenzene or *p*-monomethylaminoazobenzene.

Effect of caloric restriction.—Of all the dietary factors that have been studied in connection with carcinogenesis, caloric intake seems to have the widest effect; a reduced caloric intake decreased the rate of formation of spontaneous tumors of the lung (35) or mammary gland (36, 38), of skin tumors induced by hydrocarbons (36) or ultraviolet light (30), of subcutaneous tumors due to hydrocarbons (35, 36) and of leukemia (34) in susceptible strains of mice. Preliminary attempts have been made (7) to alter the caloric intake of rats receiving *p*-dimethylaminoazobenzene (DAB) and at the same time to insure an approximately equal intake of the carcinogen by the restricted groups and those fed *ad libitum*, since the carcinogenicity of the azo dye varies with the level of intake. Unfortunately, however, an equivalent intake of dye by the groups restricted in calories could only be achieved by increasing the percentage of azo dye in the ration, and animals fed such concentrations failed to survive.

The records of the voluntary intake of food by rats receiving azo dyes in protective or non-protective diets (Table 3) fail to reveal any constant relationship between caloric intake and carcinogenicity. When the vitamin B_6 content of the ration was varied, a decreased consumption of food (and azo dye) was associated with a decreased tumor incidence (21). An increase in the percentage of corn oil from 5 to 20 per cent resulted in a decreased caloric intake, a decreased intake of dye, but nevertheless a marked increase in tumor formation (16). On the other hand, rats fed hydrogenated coconut oil voluntarily consumed more food and carcinogen than the control animals but developed fewer tumors (8, 20, 22). The addition of riboflavin to diets containing azo dyes usually results in an increase in the intake of calories and of carcinogen while the incidence of tumors is decreased. In all of these experiments, however, dietary factors other than calories were varied, and the only conclusion that emerges is that the composition of the diet apparently exerts more effect on the development of hepatic tumors than the amount of food eaten.

However when the feeding of an azo dye is interrupted, it is possible to alter the caloric intake of groups of animals during the period of interruption and still give equal amounts of azo dye to the restricted and the control groups during the preliminary and subsequent periods. In the present experiment the restricted groups were given 62 to 63 per cent as much of the basal ration during the period of interruption as consumed by the groups fed *ad libitum*. The restriction therefore was in all nutrients present in the basal ration rather than in calories alone. Tannenbaum (37) and Rusch *et al.* (32) have shown that for other tumors the effects of restriction are essentially the same whether decreased amounts of a complete ration are fed or whether the restriction is solely in the fat and carbohydrate in the diet with an automatic increase in the percentage of all other ingredients. In the present study some restriction in food intake was also necessary after the feeding of the azo dye was resumed. The control groups were well nourished during the period of interruption, and voluntarily ate less when the feeding of azo dye was resumed. On the other hand, rats that had been partially starved during the period of interruption tended to "make up" for lost calories when given unlimited amounts of diet containing the azo dye. It was therefore necessary to equalize the intake of food and carcinogen during the first 10 to 14 days of the second dye feeding period, after which the consumption of food by the two groups was very similar.

The rats restricted in calories during the period of interruption developed significantly more tumors than the unrestricted rats of the control group. In the first series the percentage of tumors was increased from 40 in the control group to 67 in that restricted in calories during the intermediate period (Table 2, groups 19 and 20); in the second series (groups 29 and 30) the incidence was raised from 62 per cent in the control group to 93 per cent in the restricted group. Thus, in contrast to the general observation that caloric restriction decreases the development of other types of tumors caloric restriction during the intermediate period increased the development of hepatic tumors in the present experiment.

Effect of selenium.—In view of the reported carcinogenic activity of selenium (25), its ability to damage the liver (19, 24), and a possible additive effect between selenium and the azo dyes (26), 5 parts per million of selenium as sodium selenite were fed in the present experiment during a 4 week period of interruption between two 4 week periods during which 0.064 per cent *m'*DAB was fed. In two separate experiments there was a reduction of about 50 per cent in the incidence of liver tumors

when selenium was fed during the intermediate period. The incidences of tumors in the groups fed selenium and in the control groups were 22 *vs.* 40 per cent in one series and 31 *vs.* 62 per cent in the second (Table 2, groups 22 and 19, 31 and 29). The animals receiving the selenium did not gain as much weight during the intermediate period as the animals on the basal diet and during the second dye feeding period they actually gained weight as compared to a loss of weight by the animals that had received the basal diet during the intermediate period. The slower gains by the animals receiving the selenium during the intermediate period were due in part to a decrease in food intake. Subsequently when selenium was replaced by the azo dye, the consumption of food and dye by the rats previously exposed to selenium was either equal to or greater than that by the control groups. Hence the reduced tumor incidence could not be ascribed to a diminished intake of carcinogen. Furthermore the decreased intake of food during the intermediate period would have tended to increase the ultimate incidence of tumors (Table 2, groups 20 and 30). Thus the inhibiting effect of selenium on tumor developments was evident in spite of accompanying influences in the opposite direction.

Effect of 20 per cent of corn oil.—In a previous experiment tumor formation was hastened by a diet containing 20 per cent of corn oil fed simultaneously with DAB (16). In the present experiment 20 per cent of corn oil was fed during the period of interruption between two 4 week periods during which *m'*DAB was fed in the regular basal diet containing 5 per cent of the oil. All of the rats fed the higher level of corn oil developed tumors as compared with an incidence of only 62 per cent in the control group (Table 2, groups 29 and 33). Thus the high level of corn oil was approximately as effective in stimulating tumor formation when fed in the absence of the dye as in previous experiments in which a dye and the oil were fed simultaneously.

Hepatic riboflavin and coagulability as affected by diet during the period of interruption.[1]—As one approach to the means by which the diet fed during the period of interruption affects tumor incidence, fluorometric determinations (1, 5) were made of riboflavin in the livers of rats fed 0.064 per cent of *m'*DAB for 4 weeks followed by 4 weeks on the various diets under consideration. Livers from

[1] W. L. Miller, Jr., assisted in some of the analytical experiments.

TABLE 3

VOLUNTARY VARIATIONS IN CALORIC INTAKE BY RATS INGESTING AZO DYES IN PROTECTIVE AND NON-PROTECTIVE DIETS

Azo dye	Diet	Av. food consumption gm./rat/day	Av. daily caloric intake	Per cent tumors	Reference
DAB	2.5 mg./kg. B_6	11.3	43.1	92	21
"	0.2 " "	7.6	29.0	7	
"	Control	6.5	24.8	67	23
"	Vitab	7.7	29.0	100	
"	" +riboflavin	8.7	32.8	0	
"	Control	10.0	38.1	30	11
"	" low riboflavin	7.5	28.6	85	
"	Control	10.1	38.4	73	22
"	HCNO	11.3	43.1	7	
"	Low fat	11.0	39.1	8*	16
"	5% corn oil	9.7	37.0	73*	
"	20% corn oil	7.3	33.7	100*	
"	20% lard	7.8	36.0	60*	
MAB	Control	11.5	43.9	87	20
"	HCNO	13.3	50.3	31	
"	0.35% Nicotinamide	9.6	36.6	80	
*m'*DAB	Control	11.7	44.6	80	8
"	Vitab	11.0	41.7	92	
"	HCNO	12.4	47.3	53	
"	High riboflavin	11.1	42.3	47	
*o'*DAB	Control	7.3	27.8	64	8
"	Vitab	7.7	29.1	73	
"	HCNO	9.3	35.4	46	
"	High riboflavin	10.1	38.5	38	

DAB = *p*-dimethylaminoazobenzene.
MAB = monomethylaminoazobenzene.
*m'*DAB = *m'*methyl *p*-dimethylaminoazobenzene.
*o'*DAB = *o'*methyl *p*-dimethylaminoazobenzene.
HCNO = hydrogenated coconut oil.
Vitab = Rice bran concentrate.
* Tumor incidence after 4 months of dye feeding.

rats fed high amounts of casein and riboflavin contained the highest concentrations of the vitamin, 32.4 γ/gram (Table 4). Rats fed a decreased amount of food likewise contained a high concentration of riboflavin, 29.9 γ/gram of liver, although the total amount of vitamin per liver was low due to the small size of this organ. This confirms previous observations (9). The concentrations of hepatic riboflavin in the groups fed selenium or 20 per cent of corn oil during the period of recovery were essentially the same as those in the control group, 22.7 γ/gram, while rats fed nicotinamide contained relatively low concentrations of the vitamin in the liver, 17.9 γ/gram (Table 4). Feeding of the basal diet for 8 weeks after the dye feeding period only increased the concentration of riboflavin slightly (24.5 γ/gram) over that found at 4 weeks (22.7 γ/gram), and 12 weeks on the basal diet had no further effect (23.5 γ/gram).

Thus the concentration of hepatic riboflavin at the end of the period of interruption did not appear to be the primary factor affecting the ultimate incidence of tumors. The concentrations were very similar in the groups fed selenium, on which tumor incidence was retarded, and in those on a high level of corn oil on which tumor formation was accelerated. Furthermore, high concentrations of hepatic riboflavin in the group fed riboflavin and casein were associated with a doubtful decrease in tumor incidence, while on the diet restricted in calories a high concentration of riboflavin was associated with an increased incidence of tumors.

Parallel studies were made of the coagulability of homogenates from the same livers analyzed for riboflavin. Tumor homogenates or liver homogenates from rats receiving *m*′DAB for 3 or more weeks fail to coagulate upon heating, while homogenates from rats on control diets or on diets containing non-carcinogenic azo dyes coagulate in 3 to 5 minutes (10). In the present study coagulability was determined by the original method (10) except that the livers were not perfused. Liver homogenates from rats fed *m*′DAB for 4 or more

TABLE 4

HEPATIC RIBOFLAVIN AND HEAT COAGULABILITY OF LIVER HOMOGENATES FROM RATS FED *m*′DAB FOLLOWED FOR VARIOUS PERIODS BY DIETS FREE FROM DYE

DIET AND TIME		INIT. WT. gm.	FINAL WT. gm.	FOOD INTAKE on dye gm./day	FOOD INTAKE dye-free gm./day	LIVER	RIBOFLAVIN γ/liver	RIBOFLAVIN γ/gm.	COAG. MINUTE
0.064% *m*′DAB		235	180			Normal	91	16.5	
4 wks.		262	201			"	88	16.5	
		295	245			"	128	15.9	
	Av.	264	209	9.9			102	16.3	
0.064% *m*′DAB		247	201			Normal	125	16.2	35+
8 wks.		262	241			Cirrh.	146	12.9	35+
		305	246			"	119	12.5	35+
	Av.	271	229	8.7			130	13.9	35+
0.064% *m*′DAB 4 wks.		250	255			Normal	158	18.4	
Basal 2 wks.		253	263			"	167	18.9	
		325	334			"	196	21.6	
	Av.	276	264	10.6	15.0		174	19.6	
0.064% *m*′DAB 4 wks.		235	274			Normal	216	22.7	35+
Basal 4 wks.		251	272			"	200	21.6	35+
		308	300			"	220	23.9	35+
	Av.	265	282	9.2	15.5		212	22.7	35+
0.064% *m*′DAB 4 wks.		295	334			Normal	329	26.1	7
High casein and ribo-		263	280			"	425	39.3	10
flavin 4 wks.		232	292			"	344	31.8	4
	Av.	263	302	9.1	16.7		366	32.4	7
0.064% *m*′DAB 4 wks.		245	259			Fatty	236	19.7	30+
Nicotinamide 4 wks.		252	265			"	233	17.5	30+
		301	290			"	238	16.6	30+
	Av.	266	271	10.6	17.4		236	17.9	30+
0.064% *m*′DAB 4 wks.		243	209			Normal	173	18.8	30+
Selenium 4 wks.		263	212			"	163	21.4	8
		288	280			"	230	21.3	30+
	Av.	265	234	8.0	11.3		189	20.5	8–30+
0.064% *m*′DAB 4 wks.		239	197			Normal	142	29.6	3
Basal restr. 4 wks.		271	216			"	137	29.2	25
		291	219			"	166	30.8	11
	Av.	267	211	9.9	9.0		148	29.9	13
0.064% *m*′DAB 4 wks.		227	259			Normal	195	22.2	30+
20% corn oil 4 wks.		254	289			"	208	22.9	30+
		213	308			"	210	22.1	30+
	Av.	231	285	10.0	14.4		204	22.4	30+

m′DAB = *m*′ methyl *p*-dimethylaminoazobenzene.

weeks failed to coagulate on heating and this failure in coagulability was evident even after a recovery period of 4 weeks on most of the diets free from dye. Homogenates from rats fed restricted amounts of the basal ration or from those fed the diet high in casein and riboflavin coagulated in 13 and 7 minutes respectively. Since coagulation is influenced by a number of factors including the total concentration of coagulable protein (10), of fatty acids (3), or of nucleoprotein (4), the relatively rapid coagulation of livers from the restricted groups is presumably due to an altered concentration of one or more of these factors.

DISCUSSION

The intermittent application of carcinogenic hydrocarbons (31, 18), of ultraviolet light (31) or of these carcinogens and appropriate co-carcinogens (2) has previously been observed to result in a reasonably high incidence of tumors. In the present study the feeding of *m'*methyl-*p*-dimethylaminoazobenzene was interrupted for periods of 1 to 3 months and a high incidence of hepatic tumors also observed. These different types of tumors therefore are similar in that the initial application of a subcarcinogenic dose produces changes which persist for a long time, with subsequent subcarcinogenic treatments being sufficiently additive to give rise to neoplasms. Further evidence for the persistence of subcarcinogenic changes in the liver of rats fed the azo dye for 4 weeks is the fact that the failure of homogenates from such livers to coagulate on heating persisted for at least 4 weeks after the administration of dye was discontinued.

The various incidences of tumors in the groups fed different diets during the period of interruption suggest that the effects of diet previously observed need not all be ascribed to an altered metabolism of the carcinogen. The increased percentage of tumors observed when the caloric intake of the rats was restricted during the intermediate period may be related to the phenomenon of liver regeneration rather than to the caloric effect *per se* that is so important for other types of neoplasms. The livers of rats restricted in calories during the period of interruption averaged only 5.0 grams in weight as compared to 9.3 grams for the controls fed *ad libitum* during this period. During the final period of dye feeding the undersized livers increased in size, and this increase was taking place while carcinogen was present in the liver. The experiment therefore is somewhat analogous to that in which proliferation in skin exposed to hydrocarbon (17) or to a virus (27) hastens carcinogenesis.

Other nutritional effects appear to be related to the condition of the liver when the second feeding of dye was begun. Cirrhosis due to an azo dye persists longer when nicotinamide is fed than on an ordinary diet (20), and the rats fed nicotinamide during the intermediate period still had cirrhotic livers when the feeding of the carcinogen was resumed. Livers of rats fed 20 per cent of corn oil during the intermediate period did not appear cirrhotic although they were somewhat fatty when the feeding of the dye was resumed.

The other cirrhosis-producing agent studied, selenium, retarded the formation of tumors due to *m'*DAB in spite of the reported carcinogenicity of the element itself. However carcinogens are not always additive; examples of carcinogens that do not reinforce one another include hydrocarbons and ultraviolet light (29), urethane and methylcholanthrene (13), and urethane and *p*-dimethylaminoazobenzene (14). In a previous study in this laboratory no consistent additive effects between selenium and the azo dyes were observed when selenium was fed with the dye or after it (26). Attempts to demonstrate an additive effect between azo dyes and 2-acetyl-aminofluorene have likewise been unsuccessful (6).

SUMMARY

1. *m'*Methyl *p*-dimethylaminoazobenzene was fed to rats for 8 weeks, either continuously or interrupted for periods of 2 to 12 weeks. Tumors developed in the livers of all groups, the number depending on the length of the period of interruption.

2. When 0.064 per cent of the dye was fed for two 4 week periods separated by a 4 week period during which various dye-free diets were fed, the final incidence of tumors depended upon the diet fed during the intermediate period. Tumor incidence was increased by diets containing 0.29 per cent of nicotinamide or 20 per cent of corn oil or when the amount of basal diet fed during this period was restricted to 63 per cent of the calories consumed by the control group. Tumor incidence was decreased by a diet containing 5 parts per million of selenium. A diet containing 24 per cent of casein and 20 mg. of riboflavin per kg. during the period of interruption reduced the incidence of tumors in one experiment and had no effect in two others. Choline or methionine fed during the intermediate period had no effect on tumor incidence.

3. Homogenates of livers from rats fed the dye for 4 weeks failed to coagulate with heat. This decreased coagulability persisted for at least 4 weeks on most of the dye-free diets fed. The percentage of tumors that developed on the various diets did not appear to be related to the coagulability of the liver homogenates when the feeding of azo dye was resumed nor to the concentration of hepatic riboflavin at this time.

REFERENCES

1. Andrews, J. S. A Collaborative Study of Riboflavin Methods. Cereal Chem., **20**:3–23, 1943.
2. Berenblum, I., and Shubik, P. A New Quantitative Approach to the Study of the Stages of Chemical Carcinogenesis in the Mouse's Skin. Brit. J. Cancer., **1**:383–391, 1947.
3. Boyer, P. D., Lum, F. G., Ballou, G. A., Luck, J. M., and Rice, R. G. The Combination of Fatty Acids and Related Compounds with Serum Albumin. I. Stabilization against Heat Denaturation. J. Biol. Chem., **162**:181–198, 1946.
4. Carter, C. E., and Greenstein, J. P. Protective Effect of Thymus Nucleate on the Heat Coagulation of Proteins. J. Nat. Cancer Inst., **6**:219–223, 1946.
5. Conner, R. T., and Straub, G. J. Combined Determination of Riboflavin and Thiamine in Food Products. Indust. and Engin. Chem., Anal. Ed., **13**:385–388, 1941.
6. DeLeers, V. J. The Relationship of Certain Vitamins to Hepatic Tumors due to *p*-Dimethylaminoazobenzene and 2-Acetylaminofluorine. M.S. Thesis, University of Wisconsin, 1948.
7. Giese, J. E., and Baumann, C. A., Unpublished.
8. Giese, J. E., Clayton, C. C., Miller, E. C., and Baumann, C. A. The Effect of Certain Diets on Hepatic Tumor Formation Due to *m'*-Methyl-*p*-Dimethylaminoazobenzene and *o'*-Methyl-*p*-Dimethylaminoazobenzene. Cancer Research, **6**:679–684, 1946.
9. Griffin, A. C., and Baumann, C. A. The Effect of Certain Azo Dyes upon the Storage of Riboflavin in the Liver. Arch. Biochem., **11**:467–476, 1946.
10. Griffin, A. C., and Baumann, C. A. Note on the Heat Coagulation of Homogenates from Normal and Precancerous Livers. Cancer Research, **8**:135–138, 1948.
11. Griffin, A. C., and Baumann, C. A. Hepatic Riboflavin and Tumor Formation in Rats Fed Azo Dyes in Various Diets. Cancer Research, **8**:279–284, 1948.
12. Griffin, A. C., Clayton, C. C., and Baumann, C. A. The Effects of Casein and Methionine on the Retention of Hepatic Riboflavin and on the Development of Liver Tumors in Rats Fed Certain Azo Dyes. Cancer Research, **9**:82–87, 1949.
13. Jaffe, W. G. The Response of Mice to the Simultaneous Application of Two Different Carcinogenic Agents. Cancer Research, **7**:529–530, 1947.
14. Jaffe, W. G. The Response of Rats to the Simultaneous Application of Two Different Carcinogenic Agents. Cancer Research, **7**:113–116, 1947.
15. Kensler, C. J., Sugiura, K., Young, N. F., Halter, C. R., and Rhoads, C. P. Partial Protection of Rats by Riboflavin with Casein Against Liver Cancer Caused by Dimethylaminoazobenzene. Science, **93**:308–310, 1941.
16. Kline, B. E., Miller, J. A., Rusch, H. P., and Baumann, C. A. Certain Effects of Dietary Fats on the Production of Liver Tumors in Rats Fed *p*-Dimethylaminoazobenzene. Cancer Research, **6**:5–7, 1946.
17. Kline, B. E., and Rusch, H. P. Some Factors that Influence the Growth of Neoplastic Cells. Cancer Research, **4**:762–767, 1944.
18. Lavik, P. S., Moore, P. R., Rusch, H. P., and Baumann, C. A. Some Additive Effects of Carcinogenic Hydrocarbons. Cancer Research, **2**:189–192, 1942.
19. Lillie, R. D., and Smith, M. I. Histogenesis of Hepatic Cirrhosis in Chromic Food Selenosis. Am. J. Path., **16**: 223–228, 1940.
20. Miller, E. C., and Baumann, C. A. The Carcinogenicity of *p*-Monomethylaminoazobenzene in Various Diets and the Activity of this Dye Relative to *p*-Dimethylaminoazobenzene. Cancer Research, **6**:289–295, 1946.
21. Miller, E. C., Baumann, C. A., and Rusch, H. P. Certain Effects of Dietary Pyridoxine and Casein on the Carcinogenicity of *p*-Dimethylaminoazobenzene. Cancer Research, **5**:713–716, 1945.
22. Miller, J. A., Kline, B. E., Rusch, H. P., and Baumann, C. A. The Effect of Certain Lipids on the Carcinogenicity of *p*-Dimethylaminoazobenzene. Cancer Research, **4**:756–761, 1944.
23. Miner, D. L., Miller, J. A., Baumann, C. A., and Rusch, H. P. The Effect of Pyridoxin and Other B Vitamins on the Production of Liver Cancer with *p*-Dimethylaminoazobenzene. Cancer Research, **3**:296–302, 1943.
24. Munsell, H. E., DeVaney, G. M., and Kennedy, M. H. Toxicity of Food Containing Selenium as Shown by its Effect on the Rat. U.S. Dept. of Agric. Techn. Bull. No. 534, 1936.
25. Nelson, A. A., Fitzhugh, O. G., and Calvery, H. O. Liver Tumors Following Cirrhosis Caused by Selenium in Rats. Cancer Research, **3**:230–236, 1943.
26. Pearce, E. L. Dietary Factors Affecting the Resistance of Rats to Selenium. M.S. Thesis, University of Wisconsin, 1947.
27. Rous, P., and Kidd, J. G. Conditional Neoplasms and Subthreshold Neoplastic States: A Study of Tar Tumors of Rabbits. J. Exper. Med., **73**:365–390, 1941.
28. Rusch, H. P., Baumann, C. A., Miller, J. A., and Kline, B. E. Experimental Liver Tumors. A.A.A.S. Res. Conf. on Cancer. Science Press, Lancaster, Pa., 267–287, 1945.
29. Rusch, H. P., Kline, B. E., and Baumann, C. A. The Nonadditive Effect of Ultraviolet Light and Other Carcinogenic Procedures. Cancer Research, **2**:183–188, 1942.
30. Rusch, H. P., Kline, B. E., and Baumann, C. A. The Influence of Caloric Restriction and Dietary Fat on Tumor Formation with Ultraviolet Light. Cancer Research, **5**:431–435, 1945.
31. Rusch, H. P., and Kline, B. E. Influence of Interrupted Carcinogenic Treatment on Tumor Formation. Proc. Soc. Exper. Biol. and Med., **69**:90–95, 1948.
32. Rusch, H. P., Johnson, R. O., and Kline, B. E. The Relationship of Caloric Intake and of Blood Sugar to Sarcogenesis in Mice. Cancer Research, **5**:705–712, 1945.
33. Sauberlich, H. E., Pearce, E. L., and Baumann, C. A. Excretion of Amino Acids by Rats and Mice Fed Proteins of Different Biological Values. J. Biol. Chem., **175**: 29–38, 1948.
34. Saxton, J. A., Jr., Boon, M. C., and Furth, J. Observations on the Inhibition of Development of Spontaneous Leukemia in Mice by Underfeeding. Cancer Research, **4**:401–409, 1944.
35. Tannenbaum, A. The Initiation and Growth of Tumors. Introduction. 1. Effects of Underfeeding. Am. J. Cancer, **38**:335–350, 1940.
36. Tannenbaum, A. The Genesis and Growth of Tumors. 2. Effects of Caloric Restriction *per se*. Cancer Research, **2**:460–467, 1942.
37. Tannenbaum, A. Effects of Varying Caloric Intake Upon Tumor Incidence and Tumor Growth. Ann. New York Acad. Sci., **49**:5–18, 1947.
38. Visscher, M. B., Ball, Z. B., Barnes, R. H., and Sivertsen, I. The Influence of Caloric Restriction Upon the Incidence of Spontaneous Mammary Carcinoma in Mice. Surgery, **11**:48–55, 1942.
39. White, J., and Edwards, J. E. Effect of Supplementary Methionine or Choline plus Cystine on the Incidence of *p*-Dimethylaminoazobenzene Induced Hepatic Tumors in the Rat. J. Nat. Cancer Inst., **3**:43–59, 1942.

Early Changes in the Lungs of Rats Treated with Urethane (Ethyl Carbamate)

A. Rosin, M.D.*

(From the Department of Experimental Pathology, The Hebrew University, Jerusalem, Israel)

In the course of studies on the early changes in the livers of rats injected daily for several days with urethane (2), we noted proliferative changes in the epithelial elements of the lung. We decided to investigate the nature of these changes. In the meantime Nettelship, Henshaw, and Meyer (7) published observations on C3H mice treated for several months with weekly doses of urethane. They found a much higher incidence of lung tumors than in the untreated C3H mice and a much earlier appearance of the tumors than usual. The tumors, which were seen 2 to 3 months after the beginning of the treatment, were described as adenomata.

Jaffé (5) observed the same type of lung tumors as reported by the former authors in rats fed or injected with urethane after a period of more than 9 months. Guyer and Claus (4) found that intraperitoneal injections of urethane induced the formation of multiple pulmonary adenomata in the majority of 91 treated rats. Smaller foci which seemed to precede the tumors were detected as early as 8 to 10 weeks after the first treatment. Orr (9, 10) treated mice with urethane and found adenomata in the lungs of 58.4 per cent of the treated animals. The earliest adenomatous changes occurred after $2\frac{1}{2}$ months. Noble and Millar (8) injected white mice with a 25 per cent urethane solution in 5 per cent zinc acetate. They observed lung adenomas after 3 to 4 months. Besides these tumors they found lymphosarcoma in 2 mice and a malignant hemangioendothelioma in one. Selbie and Thackray (12) observed lung tumors in 100 per cent of CBA mice 7 months after intraperitoneal injection of urethane.

In our observations adenoma-like formations in the lungs of rats treated with daily injections of urethane were observed by microscopic examination as early as 4 to 5 days after the first injection. This rapid development was rather unusual and we refrained from publishing our results. We sought for adenomata in the lungs of the normal rats of our colony without, however, finding them. Recently Smith and Rous (13) reported having observed lung adenomata in newborn rats, from a mother injected with urethane several days before parturition. The tumors were seen as early as 10 days after the first administration of urethane. The early changes in the lungs of rats treated with urethane are described in the present paper.

MATERIAL AND METHODS

Albino rats weighing from 60 to 200 gms. were used for these experiments. Urethane (ethyl carbamate) in 10 per cent aqueous solution was injected intraperitoneally in doses of 1 cc. per 100 gms. body weight. Animals which did not die as a result of treatment were killed with ether. The lungs were removed, injected through the bronchi with Zenker's fluid and then fixed. Paraffin-celloidin sections 5 μ in thickness were stained with hematoxylin eosin, Giemsa's stain, and in some cases with methyl green-pyronin.

RESULTS

On gross examination the lungs of the experimental animals were often congested and edematous, but in no instance were nodules observed. Microscopic examination revealed that 6 out of 28 rats had undoubted adenomatous or papillomatous formations. Seventeen showed marked proliferation of the bronchial epithelium with downgrowth into the respiratory bronchioles and alveolar ducts. In some, proliferation of the lining cells of the pulmonary alveoli was observed. Five animals showed no changes at all.

The animals with undoubted adenomatous or papillomatous formations died or were killed 4, 5, 7, and 13 days after the first injection of urethane. The 17 rats of the second group died or were killed after 2 to 20 days. The rats which showed no changes were killed after 5 and 9 days.

Histology.—In rats treated with daily injections of urethane which died after 2 to 4 injections, *i.e.* after 2 to 3 days from the beginning of the experiment, edema was observed in some areas of the lung. Here the alveoli were filled with homogeneous pink-staining fluid. The blood vessels were surrounded by large ring-shaped spaces containing homogeneous pink fluid in which some distended

* Research Fellow of the Hebrew University.

fibers of connective tissue could be discerned. In other places the capillaries were engorged with red blood corpuscles and here and there hemorrhages were seen in the parenchyma. Sometimes slight emphysema was seen in the peripheral areas of the lobe. The mucosa of the large bronchi was swollen and hyperplastic. In a great number of bronchi there were large folds of redundant mucosa which sometimes filled the lumen of the bronchi. The bronchial epithelium, however, remained in a single layer. The epithelial cells of the bronchi contained several nuclei lying close together and forming rows. Mitoses were rare. Sometimes a few polymorphonuclear leukocytes could be seen in the lumen of the bronchi. The lining cells of the alveoli, especially of those which lie close to bronchi, were swollen and protruded into the alveolar lumina. Often numerous cells could be seen extruding into the lumen of the alveolus.

The most remarkable tumor-like formations were seen in animals which were treated with 4, 6, or more daily injections of urethane. In one animal which died 4 days after the first injection the major part of one lobe consisted of consolidated tissue containing all types of round and spindle cells and a few polymorphonuclear leukocytes. This solid tissue contained numerous papilloma-like formations apparently originating from bronchioles (Fig. 1). They were found in the neighbourhood of blood vessels and were generally lined for short stretches by a muscularis layer. Their size, however, did not correspond to the size of the vessels, since they were very large, extending into the lung parenchyma; they were not limited by a capsule. They contained long papillomatous processes which sometimes nearly obstructed the lumen. The papillae consisted of a small fibrous stroma and high cylindrical cells, often ciliated. In places the cylindrical cells seemed to extend into the alveolar ducts. This was especially noticeable on the border between the solid and air-containing tissue. Here large air spaces could be seen lined with cylindrical epithelium which slowly transformed into cubical epithelium and eventually flattened completely (Fig. 2). The alveolar septa in the air-containing tissue were rich in cells. There was no edema.

In another animal, killed 5 days after first injection of urethane, tumor-like formations were seen at different places and in different lobes. They were tubulo-papillomatous or adenomatous structures around a lumen. Some of these formations seemed to originate from respiratory bronchioles with highly proliferated mucosa showing papillomatous processes (Fig. 3). The papillae consisted of a very thin capillary loop with supporting connective tissue covered by a tuft of high cylindrical cells. The nuclei of these cells were numerous and approximately equal in size, round or oval, with fine chromatin threads and one clearly visible nucleolus. They were densely packed at the bases and in the long axes of the cells. Mitoses were scanty.

These formations were sometimes bordered for short stretches by a muscularis, while at other points no boundary was visible and there was no definite capsule (Fig. 4). Sometimes the proliferating cells invaded the neighbouring tissue, so that numerous small tubular formations could be found adjacent to one large cystoadenomatous structure (Fig. 5). These small tubules were lined with cubical cells. Between the tubules numerous free proliferating cells with clear round or oval nuclei and basophilic cytoplasm could be found. The cytoplasm stained deeply pink with pyronin. Mitoses in these cells were very numerous. The whole proliferative area contained numerous histiocytes, round cells, and fibroblasts. Polymorphonuclear leukocytes were scanty.

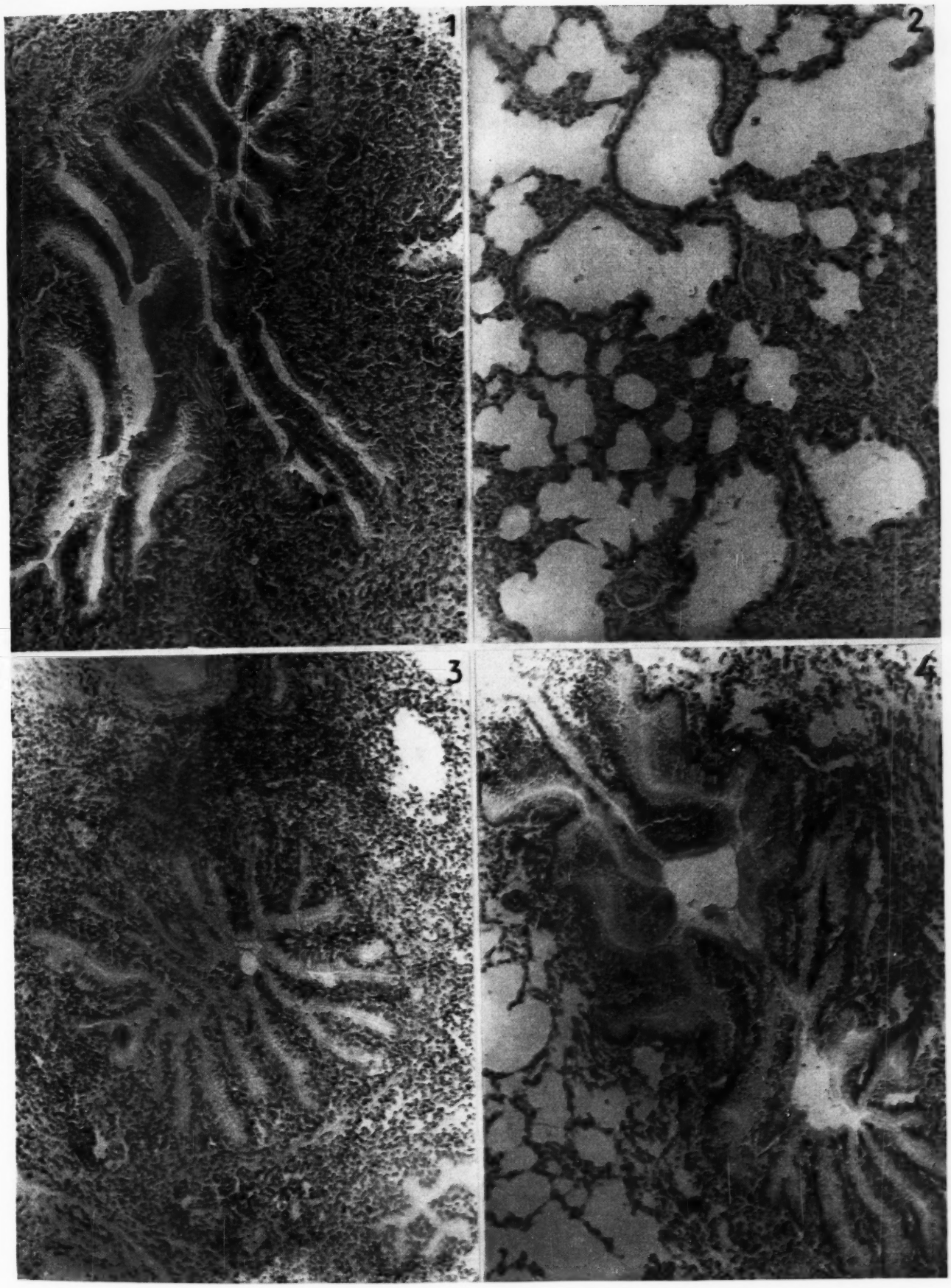
In another lobe adenomatous structures were seen. Some of them seemed to originate from bronchioles whose epithelial cells had proliferated and grown down into the alveolar ducts. Sometimes a backward growth of epithelial elements into the adjacent lung tissue could be seen. Other adenomata showed the features of alveoli invested by a continuous layer consisting of a single row of high cylindrical cells (Fig. 6). The nuclei of the cells were regular in size, round or oval, and lay in the long axis of the cell. Mitoses were not seen. There was a slight fibroblastic reaction in the surrounding tissue.

Almost all the bronchioles of the lobe showed proliferation of the mucosa. Sometimes polypous processes in the lumina of smaller bronchi were found either free or in connection with the mucosa. The respiratory bronchioles and alveolar ducts were often covered by high cubical or columna cells.

After 7 days in addition to tubulo-papillomatous formations, an intense proliferation of the lining cells of the pulmonary alveoli could be observed. There were alveoli lines with cubical cells, some of them in mitosis. In other places the alveolar ducts and alveoli were covered by high, cylindrical, mucin-secreting cells (Fig. 7). The nuclei of these cells were numerous but mitoses were scanty. Often several such adenomatous formations were found in groups and surrounded by fibroblastic tissue (Fig. 8). The picture resembled the "mucous epithelial hyperplasia" described in man by Taft and Nickerson (14). Sometimes transition of the high cylindrical cells to more cubical cells and eventually to the proliferating alveolar cells was clearly observed. The latter were very numerous. Mitoses were present.

In animals in which the changes were not so obvious an extensive downgrowth of cubical cells into the alveolar ducts could always be noted. In some animals there was intense proliferation of the mucosa of the bronchi so that their lumen was sometimes obstructed by epithelial masses.

In animals subjected to a course of treatment of



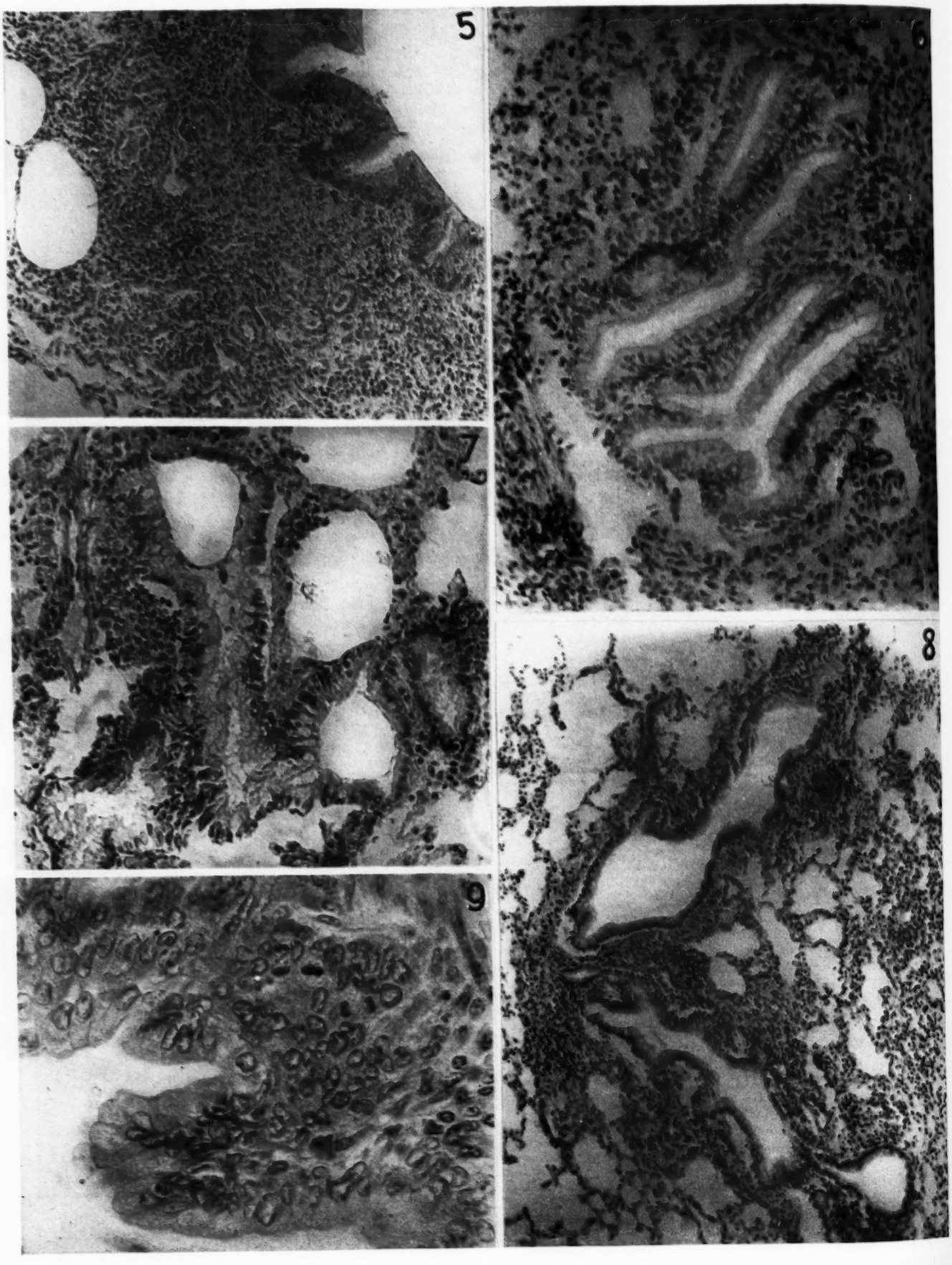
All photomicrographs were made from sections stained with hematoxylin-eosin

FIG. 1.—Rat 65, treated with 4 intraperitoneal injections of urethane on 4 consecutive days. The rat died 4 days after the first injection. Consolidated lung tissue containing papilloma-like formations originating from bronchioles. ×125.

FIG. 2.—Same rat. Border between solid and air-containing tissue. Air spaces lined with cylindrical and cubical epithelium. ×500.

FIG. 3.—Rat 129, treated with 5 intraperitoneal injections of urethane on 5 consecutive days. The animal was killed on the fifth day. Papillomatous structure bordered for short stretches by a muscularis layer. ×100.

FIG. 4.—Same rat. Adeno-papillomatous formation without boundary or capsule. ×100.



FIGS. 5 TO 9

12 to 18 days duration inflammatory processes with an abundance of polymorphonuclear leukocytes were the most prominent feature. These inflammatory processes always caused the death of the animal. Here large areas of the lung tissue were destroyed and filled with masses of leukocytes and detritus. Nevertheless there were some animals which showed proliferative changes of the above described nature. In one rat which received injections of urethane in a period of 18 days and died on the twentieth day inflammatory infiltration of the lung was found together with intense proliferation of the epithelial cells of the bronchioli which formed several layers and contained abundant nuclei often in mitosis (Fig. 9). The proliferating cells occasionally grew backwards into the peribronchial tissue. Here fibroblastic reaction was evident.

DISCUSSION

Our observations reveal that 21.4 per cent of the young adult rats treated with daily intraperitoneal injections of urethane developed adenoma- and papilloma-like formations in the lungs from 6 to 13 days after the first injection. The majority of the other treated animals without frank adenoma showed hyperplasia of the bronchial mucosa, proliferation and downgrowth of bronchial epithelium, and proliferation of the lining cells of the pulmonary alveoli.

The first changes, which could be observed as early as 48 to 72 hours after the beginning of treatment, were a conspicuous edema around the vessels, and in the pulmonary parenchyma vascular congestion and sometimes hemorrhages, and simultaneously hyperplasia and proliferation of the bronchial epithelium. The epithelial layer of the bronchi was extensively folded and in some places there were papillary processes obstructing the lumen. Here and there mitoses were seen. As early as 4 to 13 days after the beginning of treatment, besides a proliferation of all epithelial elements—bronchial as well as alveolar—tumor-like formations were seen. They were of two main types, as described also by Orr (9, 10), namely adenomatous and tubulo-papillomatous structures. In some animals both types as well as transitional stages were observed.

The adenomatous type was represented by alveoli lined with cubical or high columnar epithelium, sometimes secreting mucin. Several such formations lay together, sometimes surrounded by fibroblastic tissue. The picture recalled that described by Cowdry (1) as "Jagziekte" and by Dungal (3) as epizootic adenomatosis in sheep. A similar process was reported in man by Taft and Nickerson (15) as "Pulmonary mucous hyperplasia." Willis and Brutsaert (17) noted formations of this kind in guinea pigs treated with silica dust.

The second type showed predominantly tubulopapillomatous formations. The papillomatous villi consisted of a capillary loop with a layer of connective tissue, covered by epithelium, sometimes ciliated. These formations were sometimes bordered for short stretches by a thin muscularis layer, whereas at other points no demarcation could be seen and there was backward growth into the surrounding lung tissue. These structures were somewhat similar to the papillomata described by Magnus (6) in the lungs of mice treated with 1,2,5,6-dibenzathracene. The surrounding lung tissue was mostly collapsed and contained spindle cells, round cells, and a few polymorphs.

Regarding the histogenesis of the process, it seems that the first stage is the edema of the lung tissue followed very early by a simultaneous proliferation of the epithelial and mesenchymal elements. The proliferation of the epithelium begins in the larger bronchi, descends rapidly into the respiratory bronchioles and alveolar ducts, and eventually involves the lining cells of the alveoli. The proliferation of the mesenchymal cells seems to be a sequel to the edema.

Orr (9, 10) pointed out that the proliferation of lung epithelium after treatment with urethane is the result of a chronic inflammatory process. However, he noted the paucity of leukocytes. His earliest observations were on mice which died 76 days after the beginning of the experiment. Selbie and Thackray pointed out that the tumors observed by them in CBA mice after treatment with urethane are not preceded by pneumonic changes. Our observations suggest that the alveoli become partly filled with edematous fluid as a sequel to the continuous effect of urethane on the vascular system, and parts of the lungs are collapsed. Simultaneously proliferation of all cell elements occurs. Whether

FIG. 5.—Same rat. Numerous small, tubular formations and proliferating cells adjacent to one large, cystadenomatous structure. ×140.

FIG. 6.—Same rat. Alveoli invested by a layer of high cylindrical cells. ×200.

FIG. 7.—Rat 333, treated with 6 intraperitoneal injections of urethane on 6 consecutive days and killed on the seventh day. Alveolar ducts and alveoli covered by high cylindrical cells. ×205.

FIG. 8.—Same rat. Group of adenomatous formations surrounded by fibroblastic tissue. ×100.

FIG. 9.—Rat 536, treated with 14 intraperitoneal injections of urethane over a period of 18 days. The animal died after 20 days. Bronchiolar mucosa showing proliferation of the epithelial cells. Some cells in mitosis. ×580.

this cell proliferation is due to the influence of urethane or to some virus present in the lungs of the animals and activated by urethane, is not known. Investigations are in progress on the fate of the described epithelial proliferations, to determine whether they are permanent or reversible. The minor incidence of adenomatous formations in lungs of rats treated for 12 to 18 days with daily injections of urethane may be due to the destruction of large areas of the lung tissue by extensive inflammatory processes with abundant polymorphonuclear leukocytic infiltration.

The problem of the rapid development of the observed tumor-like formations remains. There is a very slight possibility that these changes in the lungs of the experimental animals might have occurred spontaneously, though we were never able to observe changes of a similar nature in the lungs of control rats or of hundreds of rats of the same stock treated with other substances. Dr. E. J. Farris, Executive Director and Associate in Anatomy, The Wistar Institute, Philadelphia, informed us that he has never observed these changes in large colonies of normal rats.

The changes seen by us are only microscopic foci and our experience with tissue cultures of lungs (11) leads us to believe that proliferation of epithelial cells can be very rapid. There are also some reports of rapid proliferation of epithelium in the lungs of experimental animals. Straub (14) observed proliferation of epithelial cells of the respiratory bronchioles and the alveoli with numerous mitoses in the lungs of mice infected with influenza virus as early as 5 days after the infection. Thornton and Adams (16) saw changes in the bronchial epithelium from typical to transitional epithelium as early as 5 to 7 days following application of benzopyrene in rats. Smith and Rous (12) observed adenomata in infant mice, when the mother had been treated with urethane before parturition; the adenomata were found 10 days after the first injection into the mother. The authors think that the rapid development of the tumors is due to "conditions implicit in the youth" of the cells "which already possess a natural tendency to divide." Our observations show that rapid development can also occur in young adult rats. In both cases the reduced respiration either in the days before birth or as a consequence of the deep narcosis induced by urethane may favor the proliferation of epithelium.

It must be assumed that urethane has a growth-promoting effect on the epithelial cells of the lung. On the other hand, urethane is known to be a capillary poison, augmenting the permeability of the capillary wall. The lung tissue, filled with extravasated plasma, offers a very suitable environment for the proliferation of cells as is known from many pathological conditions. Possibly this combined effect facilitates the rapid development of tumors in the lungs.

SUMMARY

Proliferation of the epithelial cells of the lung was observed in rats treated with daily intraperitoneal injections of urethane. Adenomatous and papillomatous formations were microscopically observed as early as 4 to 13 days after the first injection. The histology of these early changes is described and their histogenesis discussed.

REFERENCES

1. Cowdry, E. V. Studies on the Etiology of Jagziekte. II. Origin of the Epithelial Proliferations and the Subsequent Changes. J. Exper. Med., **42**:335–346, 1925.
2. Doljanski, L., and Rosin, A. Studies on the Early Changes in the Livers of Rats Treated with Various Toxic Agents, with Especial Reference to the Vascular Lesions. I. The Histology of the Rats Liver in Urethane Poisoning. Am. J. Path., **20**:945–959, 1944.
3. Dungal, N. Epizootic Adenomatosis of the Lungs of Sheep, its Relation to Verminous Pneumonia and Jagziekte. Proc. Roy. Soc. Med., **31**:497–505, 1937–1938.
4. Guyer, M. F., and Claus, P. E. Tumor of Lung in Rats Following Injections of Urethane (Ethyl-Carbamate). J. Cancer Research, **7**:342–345, 1947.
5. Jaffé, W. G. Carcinogenic Action of Ethyl Urethane on Rats. J. Cancer Research, **7**:107–112, 1947.
6. Magnus, H. A. Experimental Production of Malignant Papillomata of the Lung of Mice with 1-2-5-6 Dibenzanthracene. J. Path. and Bact., **49**:21–31, 1939.
7. Nettelship, A., Henshaw, P. S., and Meyer, H. L. Induction of Pulmonary Tumors in Mice with Ethyl Carbamate (Urethane). J. Nat. Cancer Inst., **4**:309–319, 1943.
8. Noble, R. L., and Millar, M. J. Some Unusual Findings on the Carcinogenic Action of Urethane in Mice. Nature, **162**:253–254, 1948.
9. Orr, J. W. Induction of Pulmonary Adenomata in Mice by Urethane. Brit. J. Cancer, **1**:311–316, 1947.
10. Orr, J. W. The Histology and Histogenesis of Pulmonary Adenomata in Mice. Brit. J. Cancer, **1**:316–322, 1947.
11. Rosin, A. Studies on Lung Tissues *in vitro* with Special Reference to the Origin of the Lining Cells of the Alveolus. Anat. Rec., **97**:447–470, 1947.
12. Selbie, F. R., and Thackray, A. C. Lung Adenomata Induced by Urethane in CBA Mice. Brit. J. Cancer, **2**: 380–385, 1948.
13. Smith, W. E., and Rous, P. The Neoplastic Potentialities of Mouse Embryo Tissues. IV. Lung Adenomas in Baby Mice as Result of Prenatal Exposure to Urethane. J. Exper. Med., **88**:529–554, 1948.
14. Straub, M. The Microscopical Changes in the Lung of Mice Infected with Influenza Virus. J. Path. and Bact., **45**:75–78, 1937.
15. Taft, E. B., and Nickerson, D. A. Pulmonary Mucous Epithelial Hyperplasia (Pulmonary Adenomatosis). A Report of Two Cases. Am. J. Path., **20**:395–411, 1944.
16. Thornton, T. F., and Adams, W. E. Metaplasia of the Bronchial Epithelium in Rats Following Application of Benzpyrene. J. Cancer Research, **4**:55–59, 1944.
17. Willis, H. St., and Brutsaert, P. Tumorlike Structures in the Lungs of Guinea Pigs Artificially Exposed to Silica Dust. Am. Rev. Tuberc., **17**:268–278, 1928.

Further Studies of the Immunological Properties of Polysaccharides from *Serratia marcescens (Bacillus prodigiosus)*

III. Passive Immunization Against the Lethal Activity of the Polysaccharides with Fractions of Mouse Antiserum Elicited by a Single Injection of Polysaccharide*

HUGH J. CREECH, PH.D., AND REED F. HANKWITZ, JR., B.S.

(From the Lankenau Hospital Research Institute and The Institute for Cancer Research, Philadelphia 30, Pennsylvania)

In previous work (1 to 4), it was demonstrated that mice could be protected against the lethal action of polysaccharides from *Serratia marcescens* by procedures of passive immunization which, in certain instances, did not seem to interfere with the tumor-necrotizing action. Moderate amounts of the serum globulin fractions from rabbits given multiple injections of the polysaccharides afforded mice substantial protection only against the lethal action of polysaccharides from the homologous strain of organism. When greater amounts of the rabbit globulin fractions were used, it was noted that the mice were protected also against the serologically unrelated polysaccharides from a different strain of organism. Globulin fractions from sera of mice given a single injection of polysaccharide were found to afford mice bearing sarcoma 37 considerable protection against the homologous polysaccharide and also, in one test, against the heterologous polysaccharide (3). Active immunization of the mouse with the polysaccharides seemed to confer an entirely non-specific protection (3). Because of the indications of lack of strain specificity in the mouse compared with the rabbit, it was considered of interest to study in greater detail the effects of passive immunization with mouse antisera. As the supplies of these particular preparations of polysaccharides are nearly exhausted and since our plans for future investigations with new preparations are different from those already described, it seemed advisable to present the following results as a concluding report of this phase of the study.

* This research was supported in part by an American Cancer Society grant recommended by the Committee on Growth of the National Research Council, and by a grant from the National Institute of Health.

EXPERIMENTAL

The preparations of the polysaccharide-lipid complex used in this work were P-10, P-10a, and P-20 from the 724 strain of *Serratia marcescens* and P-3.M and P-3.Ls from the G.W. strain. As before, these materials were supplied to us by Dr. M. J. Shear of the Chemotherapy Section of the National Cancer Institute.

Except for one special experiment, 7- to 8-week old Swiss mice from our stock colony were injected intraperitoneally with 100 γ of a preparation of polysaccharide and exsanguinated by heart puncture either 3 or 14 days later. The serum was fractionated with ammonium sulfate into the globulin and albumin components. After dialysis, determination of the protein content by micro Kjeldahl analyses, and adjustment of the protein level to 10 to 15 mg. per cc., a 1-cc. quantity of the protein in physiologic saline was injected intraperitoneally into 7- to 8-week old Swiss mice bearing sarcoma 37 which had grown to a diameter of 12 to 15 mm. Three hours later, these mice were injected intraperitoneally with 0.5 cc. of a saline solution of the amount of polysaccharide which ordinarily kills about 70 per cent of a group of tumor-bearing Swiss mice. Control groups of mice injected only with the polysaccharide, and groups injected with either bovine serum albumin or the globulin fraction from normal Swiss mice prior to the administration of polysaccharide, were included in the experimental series.

RESULTS

The observations noted in the series of tests have been compiled and summarized in the accompanying table. When 600 γ of the polysaccharide P-10a (724 strain) was administered to a

total of 85 tumor-bearing Swiss mice, it was found that 73 per cent of the mice died within 24 hours and 81 per cent died within 96 hours (line 1). This 96-hour mortality rate was assigned a ratio of 1 in the last column of the table. Prior injection of the mice with either bovine serum albumin or with the globulin fraction from blood of normal 8-week old Swiss mice at ratios of protein to polysaccharide of 16 to 25 afforded them little protection against the lethal action of the polysaccharide (lines 2 and 3). A study was also made of the influence of a passive transfer of the serum albumin fraction from mice given a single 100-γ injection of P-10. This fraction isolated from blood obtained 3 days after the injection exerted a slight protective action (line 4); when obtained at 14 days, the albumin fraction was ineffective against the lethal action of P-10a (line 5).

From lines 6 and 7, it is seen that the injection of the globulin fraction (glob. 3/P-10 and glob. 14/P-10) from sera of mice obtained either 3 or 14 days after a single injection of P-10, had a pronounced effect in decreasing the lethal activity of the polysaccharide P-10a. The globulin fraction of sera toward a different preparation (P-20) of polysaccharide from the same strain of organism appeared to be less protective when obtained on the third day than on the fourteenth day (lines 8 and 9). Prior injection with the globulin fraction from sera of mice obtained 3 days after a single 100-γ injection of the P-3.M polysaccharide (G.W. strain) afforded the mice slight protection against the lethal action of P-10a (line 10). An extensive reduction in the mortality rate was noted following the use of the globulin fraction from sera of mice obtained 10 days after the injection of 250 γ and 400 γ of P-20, spaced 7 days apart, into Swiss mice bearing sarcoma 37 (glob. S/P-20, line 11) and after injection of 100 γ and 800 γ of P-20, spaced 4 days apart, into normal Swiss mice (glob. N/P-20, line 12).

These observations were confirmed in a separate series of tests in which a 500-γ amount of P-10a was used as the lethal dose. Of the serum globulins obtained from the mice 3 days after a single injection of polysaccharide, it is seen that the globulin fraction of antiserum elicited by P-10 was highly effective and that by P-20 was only moderately effective, whereas the globulins from sera of mice injected with P-3.M showed no protective action against P-10a (lines 13 to 16).

In another series of tests in which 300 γ of the polysaccharide P-3.Ls from the G.W. strain was employed as the lethal dose, it was observed that the globulin fraction of mouse sera obtained 3 days after a single injection of 100 γ of P-3.M conferred a substantial degree of protection on the mice (lines 18 and 19). The globulin fraction of mouse sera obtained 14 days after a single injection of 100 γ of P-10 or P-20 had no significant effect (lines 20 and 21).

These results substantiate and extend our earlier findings (3, 4) that protective antibodies were elicited rapidly in the mouse by a single injection of polysaccharide. They also demonstrate that globulin fractions obtained from mouse sera following a

TABLE 1

PASSIVE IMMUNIZATION OF SWISS MICE BEARING SARCOMA 37 USING FRACTIONS OF SERA FROM SWISS MICE INJECTED WITH POLYSACCHARIDE

Line	Mouse serum protein	Poly.	Amt. in γ	Ratio: protein/poly.	No. of mice	Per cent deaths		Ratio (96 hr. mortality): expt./control
						In 24 hrs.	In 96 hrs.	
1		P-10a	600	0	85	73	81	1.0
2	Glob./normal	"	"	16–25	46	50	61	0.75
3	Bov. ser. alb.	"	"	"	34	47	65	0.80
4	Alb. 3/P-10	"	"	20	18	50	56	0.69
5	Alb. 14/P-10	"	"	"	21	57	76	0.94
6	Glob. 3/P-10	"	"	16–25	23	17	26	0.32
7	Glob. 14/P-10	"	"	"	24	17	21	0.26
8	Glob. 3/P-20	"	"	"	28	25	43	0.53
9	Glob. 14/P-20	"	"	"	31	19	19	0.23
10	Glob. 3/P-3.M	"	"	"	42	43	48	0.59
11	Glob. S/P-20	"	"	16	10	10	10	0.12
12	Glob. N/P-20	"	"	"	20	10	15	0.19
13		"	500	0	16	31	50	1.0
14	Glob. 3/P-10	"	"	20	9	0	0	0
15	Glob. 3/P-20	"	"	20	8	0	25	0.5
16	Glob. 3/P-3.M	"	"	20	16	38	50	1.0
17		P-3.Ls	300	0	30	63	77	1.0
18	Glob. 3/P-3.M	"	"	50	14	29	29	0.38
19	Glob. 3/P-3.M	"	"	100	10	30	30	0.39
20	Glob. 14/P-10	"	"	50	8	50	63	0.82
21	Glob. 14/P-20	"	"	50	9	67	67	0.87

single injection of P-10 polysaccharide afforded mice a high degree of protection against the lethal action of that polysaccharide at relatively low globulin to polysaccharide ratios of 16 to 25. Similar extents of protection against P-10 were noted previously only with globulin to polysaccharide ratios of about 100 when the γ-globulin fractions of rabbit antisera elicited by a series of injections of P-10 were employed (3).

In addition, the serum globulin fractions from mice given a single injection of the polysaccharides exhibited a degree of specificity similar to that observed with globulin fractions from rabbit antisera elicited by multiple injections (2, 3). Thus, at low globulin to polysaccharide ratios, there was no significant cross protection between the polysaccharides from the 2 strains (lines 10, 16, 20, and 21) whereas at higher ratios of about 100, there was cross protection (3, Table 3) with mouse as well as rabbit globulin fractions from antisera toward the polysaccharides (2, 3).

SUMMARY

It has been found that passive immunization with relatively small amounts of the globulin fractions obtained from sera of mice either 3 or 14 days after a single injection of polysaccharides from *Serratia marcescens* afforded mice bearing sarcoma 37 pronounced protection against the lethal action of the homologous polysaccharide but not against that of the polysaccharides from a different strain of the organism.

REFERENCES

1. CREECH, H. J., HAMILTON, M. A., and DILLER, I. C. Comparative Studies of the Immunological, Toxic and Tumor-Necrotizing Properties of Polysaccharides from *Serratia marcescens* (*Bacillus prodigiosus*). Cancer Research, **8**:318–329, 1948.
2. CREECH, H. J., HAMILTON, M. A., NISHIMURA, E. T., and HANKWITZ, R. F., JR. The Influence of Antibody-Containing Fractions on the Lethal and Tumor-Necrotizing Actions of Polysaccharides from *Serratia marcescens* (*Bacillus prodigiosus*). Cancer Research, **8**:330–336, 1948.
3. CREECH, H. J., HANKWITZ, R. F., JR., and WHARTON, D. R. A. Further Studies of the Immunological Properties of Polysaccharides from *Serratia marcescens* (*Bacillus prodigiosus*). I. The Effects of Passive and Active Immunization on the Lethal Activity of the Polysaccharides. Cancer Research, **9**:150–157, 1949.
4. WHARTON, D. R. A., and CREECH, H. J. Further Studies of the Immunological Properties of Polysaccharides from *Serratia marcescens* (*Bacillus prodigiosus*). II. Nature of the Antigenic Action and the Antibody Response in Mice. J. Immunol., 1949. In press.

American Association for Cancer Research, Inc.

40th Annual Meeting

Hotel Fort Shelby, Detroit, Michigan

April 16 and 17, 1949

Proceedings of Scientific Sessions

A SEROLOGICAL FLOCCULATION REACTION AS AN INDICATION OF MALIGNANCY.

H. S. Penn. (Department of Zoology, University of California, Los Angeles, Cal.)

When the serum of a patient suffering from malignancy is mixed with the unsaponifiable fraction obtained from human cancerous liver, there is noted in a majority of instances (between 80 to 98 per cent) a flocculation characterized by the appearance of particles, and partial or complete clearance of the opaque mixture. This flocculation occurs only rarely (about 1 per cent) when the serum of healthy individuals is employed, and to a limited extent (20 to 25 per cent) in other pathological conditions. The phenomenon is not observed when the lipoid of non-cancerous livers is used as an antigen.

This investigation was based on the hypothesis of the formation of specific antibodies in response to an endogenous carcinogen, presumably steroid in nature. A complete description of the preparation of the antigen and the method of application is given. Approximately 3,000 sera were tested. Of these, 312 biopsy-proven positive sera were submitted as unknowns. Correct diagnoses were made in 259 cases (83 per cent). The accuracy of the flocculation reaction seems to vary with different malignancies, cancer of the skin being the least reliable (about 70 per cent), whereas bronchogenic carcinoma has given about 98 per cent correct diagnoses.

HETEROLOGOUS OCULAR TRANSPLANTATION AS A PRACTICAL TEST FOR CANCER.

John A. Schilling, Albert C. Snell, Jr., and Benedict V. Favata. (Department of Surgery, The University of Rochester School of Medicine and Dentistry, Rochester, N.Y.)

Carefully selected fragments from 36 proven human cancers were transplanted into the anterior chamber of 368 eyes in 218 guinea pigs. Growth occurred in eight of the 36 cases in 29 eyes of 20 guinea pigs. Growth occurred in 15 of 19 of these cases when the same human tissue was studied simultaneously by routine tissue culture *in vitro*. Growth was delayed as long as four months after intraocular transplantation, necessitating an arbitrary period of at least six months' observation before considering the result final. When growth occurs after transplantation into the anterior chamber of the eye of a heterologous host, according to well defined criteria, it is definite evidence of a high degree of autonomy and may be helpful in gaining insight into the growth potential of a given neoplasm. The method is not reliable for the clinical diagnosis of cancer because of the high incidence of failures (78 per cent) of growth in proven malignancy.

THE TISSUE TRANSPLANT TECHNIC AS A MEANS FOR TESTING MATERIALS FOR CARCINOGENIC ACTION.

William E. Smith, M.D. (Sloan-Kettering Institute for Cancer Research, New York 21, N.Y.)

Induction of cancers from epithelial tissues of mouse embryos has been previously described. The method has been to transplant pieces of selected organs together with methylcholanthrene into adult hosts of an inbred strain of mice. Neoplastic potentialities of various tissues were thus explored.

The method has now been extended with a view to employing it as a means of testing materials for carcinogenic action. Olive oil, the solvent used in previous tests, has been replaced by tricaprylin. Cancers have been readily induced from fragments of mouse embryo skin transplanted with equimolar (0.02M) solutions of 20-methylcholanthrene, 3,4-benzypyrene or 1,2,5,6-dibenzanthracene. Each of these solutions elicited squamous cell cancers or malignant papillomas at practically every implant site: within 45 to 73 days with methylcholanthrene, within 108 to 175 days with the other two carcinogens.

To adapt the method for study of weak carcinogens, experiments were carried out with a barely effective (0.01M) concentration of dibenzanthracene with and without addition of 2 per cent Scharlach R and 0.1 per cent croton oil. These latter substances, not in themselves carcinogenic, increased the yield of cancers.

This technic is being employed for assaying carcinogenicity of chemicals available only in small amounts. Tests have also been carried out with tissues available in only small amounts. A transplantable carcinoma has been induced from fragments of thyroid tissue taken from a young adult mouse and transplanted with methylcholanthrene.

FACTORS INFLUENCING THE STABILITY OF CULTURED STRAINS OF NORMAL AND MALIGNANT CELLS.

George O. Gey and Frederik B. Bang (by invitation). (The Johns Hopkins University Medical School, Baltimore 5, Md.)

Prolonged cultivation of normal and malignant cells has revealed a wide degree of variation occurring within some of the stocks while others remain fairly stable. Many of the normal and malignant strains to be described have been under cultivation for from 10 to 18 years. Some of the normal strains have become permanently altered *in vitro* and others have become malignant. Some malignant strains of host origin show great uniformity in their physiological and morphological characteristics. Others have shown profound changes after prolonged cultivation. The factors which presumably can alter the stability of cells *in vitro* will be discussed. They include viruses, hormones, and nutritive conditions. The study also emphasizes the comparative importance of homologous and heterologous media for the maintenance of some types of cells, and for the study of virus-host cell relationships.

Media sterilized with fast electrons (Capicitron) or with ultraviolet (2537) have been successfully used for cell cultivation in order to eliminate virus contaminants. This method may prove important for the study of specific viruses grown with cells and for studies with the electron microscope.

A STUDY *IN VITRO* OF A STRAIN OF LYMPHOBLAST-LIKE CELLS, MB13, DERIVED FROM MOUSE LYMPHOSARCOMA MB (T86157).

Willemina M. de Bruyn. (Division of Cell Physiology, Department of Surgery, The Johns Hopkins Hospital and School of Medicine, Baltimore 5, Md.) (Introduced by George O. Gey.)

Previous investigations have shown that the malignant lymphoblasts of lymphosarcoma MB could be maintained in continuous roller tube culture indefinitely, but only in the presence of growing mesenchymal cells. A few cultured cells can produce a tumor and kill in 16 days.

Cultivation of malignant lymphoblasts without fibroblasts was attempted by varying the media and the temperature. In several tubes in one experiment the fibroblasts died out, leaving only masses of active lymphoblast-like cells. They had been grown in chicken plasma, human cord serum, bovine embryo extract, and balanced salt solution. A strain of lymphoblast-like cells, MB13, was developed from one of these tubes. These cells vary much in size and shape. They now grow without fibroblasts and without plasma. They need embryo extract and grow best when only part of the medium is replenished with fresh fluid. Four months after the change in the cells had taken place, they still produced a tumor. From then on all injections were unsuccessful. Since these cells had been cultivated in an entirely heterologous medium for several months, experiments are under way to find out if the MB13 cells have lost their ability to grow in the mouse. The following experiments were attempted:

a. Re-establishment of mouse fibroblasts and MB13 cells in a heterologous medium.

b. Cultivation of MB13 cells, but without fibroblasts, in a medium with mouse embryo extract replacing bovine embryo extract.

To date lymphoblast-like cells, MB13, have failed to grow with mouse mesenchymal cells, whereas they grow equally well in media with mouse embryo extract as in media with bovine embryo extract.

THE EFFECT OF REGENERATION ON THE GROWTH CAPACITY OF RAT LIVER *IN VITRO*.

André D. Glinos. (From the Division of Cell Physiology, Department of Surgery, Johns Hopkins Hospital and Medical School, Baltimore, Md.)

The present work is the first part of a study aiming to investigate further the relationship between liver regeneration and carcinogenesis with *p*-dimethylaminoazobenzene. The roller tube tissue culture method of Gey was used in devising a test for the growth capacity *in vitro* of normal rat liver and the effect of regeneration upon it. The following experimental procedure was used on each animal: a) partial hepatectomy, b) immediate explantation of part of the removed median lobe, c) explantation, 72 hours post-operatively, of part of the remaining right lateral lobe.

It was found that the growth capacity of normal rat liver is an inverse proportional function of the age of the animal. In the case of young rats, 4 to 8 weeks old, 10 out of 10 or 100 per cent of the explanted livers grew *in vitro;* in adult rats, 4 to 8 months old, 5 out of 11 or 45 per cent; in old rats, 18 to 24 months old, 1 out of 12 or 8 per cent.

Regeneration had a marked effect on this relationship between age and growth capacity *in vitro*. In the young rats 7 out of 7 explanted regenerating livers grew *in vitro;* in the adults 11 out of 11; in the old rats 8 out of 8. Thus the growth capacity of regenerating liver, 72 hours after partial hepatectomy, was 100 per cent throughout the age groups and therefore independent of the age of the anima[1].

Differences in the morphology and organization of the colonies of normal and regenerating liver and their relationship to age have also been established.

CERTAIN RECENTLY DEVELOPED TISSUE CULTURE PROCEDURES APPLICABLE TO STUDY OF THE NUTRITION OF NORMAL AND MALIGNANT CELLS.

W R. Earle, K. K. Sanford, V. J. Evans, E. L. Schilling (by invitation), and G. D. Likely (by invitation). (National Cancer Institute, Bethesda 14, Md.)

This paper summarizes a group of recently developed procedures by which a strain of sarcoma cells has been grown from a single cell, and by which cel's may be grown in tissue culture under sheets of perforated cellophane instead of embedded in a plasma matrix of unknown and variable composition.

When so grown the cells can be scraped off from the cellophane and made up as a cell suspension which can be used to plant up to 200 or more replicate cultures

from one uniform inoculum suspension. By a special hemocytometer technique the actual number of cell nuclei in each unit volume of the original cell suspension planted can be determined. By sacrificing individual cultures from a series, changes in the number of nuclei present may be accurately followed. The procedures seem particularly well adapted to proliferation studies of the nutrition of the malignant cell and of the normal cell from which it arose.

In the instance of rapidly growing cell strains, using these cellophane procedures, dense sheets of cells covering areas of 70 square centimeters can be obtained in a few days. While the largest sheet so far obtained had an area of only about 190 square centimeters, work is now in progress to further adapt the method for practicable routine growth of far larger cultures.

THE LETHAL MUTATION RATE IN *DROSOPHILA MELANOGASTER* FOLLOWING THE ADMINISTRATION OF 20-METHYLCHOLANTHRENE AND METHYL-*BIS*(BETACHLOROETHYL)AMINE HYDROCHLORIDE.* WALTER J. BURDETTE. (School of Medicine, Louisiana State University, New Orleans, La.)

There is some evidence that the carcinogen, 20-methylcholanthrene, is also mutagenic in mice. Since it is highly important to know if these two properties are related, it is desirable to test the mutagenic activity of carcinogens in a form which is more suitable for the detection of such changes. Using the Muller-5 and Oregon-R stocks of *Drosophila melanogaster* tests were made for lethals on the x-chromosome. The females were treated with 20-methylcholanthrene in sesame oil by the vaginal douche technic. This was continued serially for 12 generations during a period of 148 days. The wild type chromosomes tested each successive generation had therefore been treated from 1 to 12 times. Moreover for the last 10 generations the sc^8 w^a *B* chromosomes treated twice were also tested. Three lethals on the sc^8 w^a *B* chromosome, one visible mutation to *y*, and one instance of non-disjunction were found among the 4,660 chromosomes tested. It is apparent that 20-methylcholanthrene was not mutagenic in these studies although the same material was carcinogenic for C3H mice. On the other hand the mutation rate for these stocks was increased simply by dropping methyl-*bis*(betachloroethyl)amine hydrochloride on the abdomen. In one such experiment there were 15 lethals among 2,494 chromosomes tested. The results with methylcholanthrene may be explained either by assuming it is not mutagenic or that it is strain limited or not effective by the route and concentration used. This should make one cautious in interpreting isolated instances of mutations as necessarily being due to concomitant treatment with carcinogen.

POLYCYTHEMIA ASSOCIATED WITH A TRANSPLANTABLE LUTEOMA. RAYMOND G. GOTTSCHALK and JACOB FURTH. (Veterans Administration Hospital and Southwestern Medical College, Dallas, Texas)

* This investigation was supported by a grant from the National Cancer Institute, U.S. Public Health Service.

Repeated blood volume and hematocrit determinations were made on mice by means of a microtechnic requiring less than 0.1 cc. of blood. The occurrence of hypervolemia with grafted granulosa cell tumors has been confirmed. The increase of blood volume is mainly due to an increase of the plasma volume, with little variation of the total volume of red cells, and with a relative anemia. Transplantable luteomas are associated with polycythemia, the number of red cells rising as much as 50 per cent; the hematocrit values are elevated by as much as 30 per cent. Luteoma bearing mice generally have but a mild hypervolemia due to the increase of erythrocytes, the plasma volume remaining about constant. The most severe polycythemia was found in cases of intrahepatic graft of the tumor.

These observations recall data indicating that estrogens decrease and androgens and adrenal cortical hormones increase the number of red cells. The granulosa cell tumors produce estrogens, while the luteomas are masculinizing. The hematologic changes secondary to these ovarian tumors seem related to the influence of sex hormones on erythropoiesis. The mice with transplanted luteomas show most of the elements of Cushing's syndrome, including polycythemia; this syndrome is also associated with the so-called adrenal rest tumors of the ovary in human patients.

CONTRASTING EFFECTS OF FOLIC ANTAGONISTS AND NITROGEN MUSTARDS ON LEUKEMIC CELLS *IN VIVO*.* J. H. BURCHENAL,† M. A. CREMER, and B. S. WILLIAMS (Introduced by C. P. RHOADS.) (Section on Mouse Leukemia of the Division of Experimental Chemotherapy, Sloan-Kettering Institute for Cancer Research, New York 21, N.Y.)

A secondary screening technique has been used to study compounds which have previously shown the ability to prolong the survival of mice with transmitted leukemia. In this method advantage has been taken of the delayed toxicity even at high doses of certain of these agents in examining the short term effects of supralethal doses in inactivating leukemic cells *in vivo*.

Donor mice having enlarged spleens from a generalized leukemia or with localized tumors from injection of the leukemic suspension by the subcutaneous route were injected intraperitoneally with various multiples of the LD_{50} dose of a given compound. Two hours later or immediately after death if the drug at very high doses was lethal in less than this time, the spleens or tumors were removed under aseptic precautions, minced in saline, and injected intraperitoneally into recipient mice of the same inbred stock for bioassay. At least 2 donor mice were used at each level and the leukemic suspension from each donor was bioassayed into 4 mice.

With the nitrogen mustards tested, a cytocidal or inactivating dose was found to occur in the range between

* This investigation was supported (in part) by a research grant from The National Cancer Institute of The National Institute of Health, U.S. Public Health Service, and (in part) by a research grant from The American Cancer Society.

† Fellow of The American Cancer Society, recommended by the Committee on Growth of The National Research Council.

four and ten times the LD_{50}. With 4-amino-pteroyl glutamic acid, 4-amino-N10-methyl-pteroyl glutamic acid, and 2,6-diaminopurine, however, the bioassays were positive up to and including one hundred times the LD_{50}. Even after exposure for 48 hours to 20 times the LD_{50}, no inactivating effect was seen.

THE INFLUENCE OF PROGESTERONE ON MAMMARY TUMORS INDUCED IN RATS BY ACETAMINOFLUORENE. A. Cantarow, K. E. Paschkis, and J. Stasney. (Jefferson Medical College, Philadelphia 7, Pa.)

Data previously reported from this laboratory indicated that administration of progesterone greatly increased the incidence of mammary carcinoma in female Sherman and Wistar rats receiving 2-acetaminofluorene. Estradiol was apparently without influence in intact rats. The possibility was suggested that whereas small amounts of estrogen are probably necessary for the development of breast cancer in rats receiving AAF, the quantity of progesterone may be the limiting factor in this connection. The present experiments represent an attempt to test this hypothesis.

(a) Progesterone was administered to castrate male and female rats, (b) progesterone and estradiol to intact males, (c) progesterone and estradiol to castrate males and females, (d) progesterone and estradiol to intact females, and (e) progesterone and testosterone to intact males.

No breast tumors developed in progesterone-treated intact males and castrate males and females, nor in progesterone-testosterone-treated males. One tumor developed in 10 intact males receiving estradiol and progesterone. Two tumors appeared in 33 castrate males receiving estradiol and progesterone. Four tumors appeared in 20 castrate females receiving estradiol and progesterone. One tumor appeared in 24 intact female rats receiving estradiol and progesterone. This is in sharp contrast to the 85 per cent incidence of breast tumors in such rats receiving progesterone alone and the 25 per cent incidence in intact females receiving no hormone. The significance of these observations is discussed.

STEROID EXCRETION IN GASTRIC CANCER. K. Dobriner, S. Lieberman (by invitation), J. Abels (by invitation), F. Homburger, E. C. Reifenstein, Jr., and C. P. Rhoads. (Sloan-Kettering Institute for Cancer Research, New York 21, N.Y.)

Gastric cancer is one of the most important types of neoplastic disease not only because of its high incidence but also because early diagnosis presents so many difficulties. These considerations led us to investigate the steroid excretion patterns in 2 men and 2 women suffering from advanced gastric cancer. In all of these patients a metabolite of adrenal cortical origin, 11βhydroxy-etiocholanolone was identified. This same compound was previously found in a significant number of patients with neoplastic disease and was rarely present in the urine of normal individuals (Dobriner, Lieberman, and Rhoads, Cancer Research, 7:711, 1947). Two other compounds of adrenal cortical origin, 11βhydroxyandrosterone and 11-ketoetiocholanolone, were found in the urine of all 4 gastric cancer patients. These substances are regular constituents of the urine but the amount excreted by the gastric cancer patients was smaller than that usually present in normal subjects. Androsterone and etiocholanolone, both constituents of the urine of normal people were either absent or present in very small amount in the gastric cancer patients. The pattern of ketosteroid excretion in gastric cancer resembles those obtained in prostatic cancer. The clinical significance of steroid metabolism in relation to adrenal cortical function will be discussed.

THE EFFECT OF TESTOSTERONE PROPIONATE THERAPY ON THE EXCRETION OF HORMONES IN PATIENTS WITH METASTATIC BREAST CARCINOMA.* Albert Segaloff, Richard L. Coppedge (by invitation, and J. V. Schlosser (by invitation). (Departments of Medicine and Physiology, Tulane University, the Alton Ochsner Medical Foundation, and the Charity Hospital of Louisiana, New Orleans, La.)

Seven patients have been studied to date in whom it has been possible to obtain excretion studies before and during testosterone propionate therapy for carcinoma of the breast with bone metastases. Six of the 7 patients showed the expected increase in 17 keto-steroids while on testosterone propionate therapy. Androgen bioassays have been completed on one patient and this patient also showed an increase in androgen excretion. One patient had no detectable gonadotrophic hormone before therapy, probably due to her advanced cachectic state. The others showed normal or elevated urinary gonadotrophic hormone and showed the expected decrease in its excretion while on therapy.

Cortin excretion was measured by its ability to deposit hepatic glycogen in adrenalectomized mice. This was studied in 6 patients, 3 of whom showed a marked increase in excretion while on therapy. The other 3 showed little or no change. The urinary excretion of lactogenic hormone was studied in 4 patients, 3 of the 4 showing an increase in excretion.

Clinically, 5 of the 7 patients had a marked improvement in course while on testosterone therapy, most of them gaining weight and having freedom from pain. The other 2 apparently had little or no effect on the course of their disease as the result of therapy with testosterone propionate. The testosterone propionate therapy was given as intramuscular injections of 100 mg. every other day.

INFLUENCE OF AGE OF HOST AND OF OVARIES ON TUMORIGENESIS IN INTRASPLENIC AND INTRAPANCREATIC OVARIAN GRAFTS. W. U. Gardner and M. H. Li (by invitation). (Department of Anatomy, Yale University School of Medicine, New Haven, Conn.)

Four groups of experiments were undertaken to determine the effect of age of the ovary and age of the host

* This investigation was supported by a research grant from the National Cancer Institute of the National Institute of Health, U.S. Public Health Service.

on experimental ovarian tumorigenesis. (a) Ovaries from young mice were transplanted intrasplenically into young mice. (b) Old mice (204 to 370 days) were castrated and ovaries from animals of similar ages implanted intrasplenically. (c) Ovaries from old mice (304 to 491 days) were transplanted intrasplenically or intrapancreatically into young castrated mice.

The age of the host was more important than the age of the donor; hosts showed a high incidence of ovarian tumors, granulosa cell and luteomas, irrespective of the age of the ovarian transplant at the time of grafting. Both intrasplenic and intrapancreatic transplants of ovaries into castrated mice became tumorous. Mice of all inbred strains used, one not studied previously in such experiments, showed similar responses. The tumors arising from the transplants of old ovaries were not unlike those arising from transplants of young ovaries.

These experiments indicate that ovarian granulosa cell tumors and luteomas can arise from the aged ovary and that the humoral environment of the younger animal is more favorable.

SEX HORMONE SECRETION BY ADRENAL CORTICAL TUMORS OF MICE. MARTHELLA J. FRANTZ (by invitation), and ARTHUR KIRSCHBAUM. (Department of Anatomy, University of Minnesota Medical School, Minneapolis, Minn.)

The type of sex hormones secretion in gonadectomized mice bearing cortical adenomas was determined by histologic study of submaxillary glands, renal corpuscles, and reproductive tracts. Animals of both sexes of the NH and Bagg albino strains, and females of the C3H, CBA and Strong A strains were studied.

Adenomas which secreted only estrogenic hormone were present in NH mice 70 or more days following gonadectomy. Similar tumors (10 months post-operatively) of the Bagg albino stock secreted primarily androgenic hormone. The seminal vesicles of most males castrated 2 years before autopsy were developed and secreting; submaxillary glands and kidneys were masculinized. Ten months following ovariectomy submaxillary glands of CBA and C3H females were masculinized, whereas the vaginal epithelium was cornified, indicating simultaneous secretion of estrogen and androgen. In the Strong A strain, the reproductive tract was atrophic, although submaxillary glands were male, 14 to 16 months following ovariectomy, indicating secretion of only androgen.

Hormonal secretion of cortical tumors was characteristic for the strain. The earlier tumors developed following gonadectomy, the greater the tendency towards estrogenic activity. Where both sex hormones were secreted simultaneously 10 months following gonadectomy as in the CBA and C3H strains, primarily estrogenic activity was detected from 6 to 8 months postoperatively. In the Strong A strain where the latent period of tumor formation was relatively long, cortical tumors demonstrated only androgenic activity.

Cessation of gonadal endocrine secretion was not essential for the development of spontaneous cortical tumors in both sexes of the NH and Bagg albino strains.

LEYDIG CELL TUMORS INDUCED EXPERIMENTALLY IN THE RAT. GRAY H. TWOMBLY, DORIS MEISEL (by invitation), AND ARTHUR PURDY STOUT. (College of Physicians and Surgeons, Columbia University, New York 32, N.Y.)

Fifty young adult castrated female rats were treated by the transplantation of a testis into the spleen. The testes were taken from day old rats and the implantation was done beneath the capsule of the spleen with a trochar. A similar group of 50 male castrates and a control series of 25 normal males were prepared in the same way. The animals died or were sacrificed after 240 to 450 days. In the castrated females 20 Leydig cell tumors were found; the largest was 5 cm. in diameter. Sixteen tumors occurred in the 50 castrated males. While histologically some of these tumors appeared malignant, no metastases were found. In the control males the remains of atrophic testicular tissue could be seen but no Leydig cell tumors occurred. All stages in the development of these neoplasms from Leydig cell hyperplasia through adenoma formation to what appeared to be malignant tumors were easily seen in this material. Some of the tumors are histologically remarkably like human Leydig cell tumors with which we have compared them.

TUMORS OF PITUITARY AND TRACHEA IN MICE AFTER HIGH DOSAGES OF RADIOACTIVE IODINE. AUBREY GORBMAN. (Department of Zoology, Barnard College, Columbia University, New York, N.Y.) (Introduced by E. D. Goldsmith.)

About ten months after single injections of 4 to 50 millicurie-per-kilogram dosages of I^{131} tumorous enlargements of hypophyses were found, as well as fibrous tumors of the tracheal tunica propria. The hypophyseal growths, as much as 80 times larger than the normal pituitary, contained patches of normal acidophils, and very few normal basophils. Their bulk was made up of enlarged highly vacuolated cells. Visual and motor disturbances were frequent external manifestations in mice bearing hypophyseal tumors.

The tracheal growths were fibrous in their bulk, but covered with a metaplastic simple or stratified squamous epithelium. This epithelium in some instances was extremely active mitotically. By reduction of the tracheal lumen such tumors resulted in labored respiration and apparently contributed to the death of several animals.

EFFECT OF REVERSED RESTRICTION ON MAMMARY TUMOR INCIDENCE IN OVARIECTOMIZED C3H MICE. CARMEN V. CASAS (by invitation), JOSEPH T. KING (by invitation), and M. B. VISSCHER. (Department of Physiology, University of Minnesota, Minneapolis, Minn.)

The effect of *ad libitum* feeding and caloric restriction on the incidence of mammary tumors in castrate G3H female mice has been tested by reversing type of feeding at various intervals as indicated below. The details of the diet and housing conditions have been published previously by Visscher *et al.* The restricted mice received normal amounts of protein, vitamins and min-

erals. Ovariectomy was performed at weaning and the mice were immediately placed on the diet indicated. The number of mice in each group and the number of tumors occurring through the fourteenth month are shown below.

Feeding	Tumors
Full-fed 14 mos.	15/20
" " 4 mos., then restricted	5/10
" " 3 mos., " "	3/10
" " 1 mo., " "	0/10
Restricted 14 mos.	1/10
" 7 mos., then full fed	3/10
" 4 mos., " " "	3/10
" 2 mos., " " "	3/10
" 1 mo., " " "	6/10

ISOLATION, PROPERTIES AND CHEMISTRY OF *ALPHA* AND *BETA*-PELTATIN. J. L. Hartwell (by invitation), and W. E. Detty (by invitation). (Chemotherapy Section, National Cancer Institute, Bethesda 14, Md.)

Podophyllin (N.F.) has been shown (Hartwell and Shear, Cancer Research, **7**:716, 1947; unpublished work of Leiter *et al.*) to contain at least 3 pure, colorless, crystalline components which together account for the major part of the tumor-damaging activity of the whole drug. Of these, podophyllotoxin is well-known while *alpha* and *beta*-peltatin are of recent discovery (Hartwell, J. Am. Chem. Soc., **69**:2918, 1947; Hartwell and Detty, J. Am. Chem. Soc., **70**:2833, 1948).

These compounds are concentrated in the hexane-insoluble chloroform-soluble fraction which is a brown resin amounting to about 50 per cent of the original drug. This fraction is dissolved in a benzene-alcohol mixture and chromatographed on activated alumina; podophyllotoxin, *beta*-peltatin, and *alpha*-peltatin pass thru the tower in that order. The yields are about 7, 5, and 6 per cent, respectively.

The peltatins form colorless crystals soluble in many organic solvents (including alcohol and chloroform) and in dilute solutions of caustic alkalies, but insoluble in petroleum ether and water. Both melt, without sharp m. p., around 230, and both are levorotatory ($[\alpha]_d^{20} = -115°$ in alcohol).

The peltatins are isomeric with podophyllotoxin, $C_{22}H_{22}O_8$. All three have a methylenedioxy group, and a lactone group. *Alpha*-peltatin has two phenolic hydroxyl and two methoxyl groups, while *beta*-peltatin has one phenolic hydroxyl and three methoxyl groups. Each peltatin gives rise to a diastereoisomer when treated with alkaline reagents, and to two sets of acetates and methyl ethers depending on conditions of formation. With each peltatin one set of derivatives is identical with the corresponding ones prepared from its diastereoisomer.

Evidence is presented, from ultra-violet absorption and oxidation studies, for considering the peltatins to be structurally related to podophyllotoxin.

THE ACTION OF SUBSTANCES EXTRACTED FROM PODOPHYLLIN ON SARCOMA 37 IN MICE. J. Leiter, V. Downing (by invitation), J. L. Hartwell (by invitation), and M. J. Shear. (National Cancer Institute, Bethesda 14, Md.)

A single subcutaneous injection of 10 to 50 micrograms per gram of body weight of the crude drug podophyllin produced softening, hemorrhage, and necrosis in sarcoma 37. The microscopic effects, previously described by MacCardle and Downing (1947) and 1948, were marked pycnosis of the tumor cells as well as arrest in mitosis. Similar effects were noted with a single injection of 3 crystalline components isolated from the crude drug, *viz.*, podophyllotoxin, *alpha*- and *beta*-paltatin. Quercetin, another known component in podophyllin, produced no such effects at doses up to 1000 micrograms per gram. Picropodophyllin and the sodium salt of podophyllic acid produced no visible gross effects and no pycnosis at doses up to 500 and 2000 micrograms per gram respectively, although both produced distorted mitotic figures.

The minimum effective dose (MED) of podophyllotoxin, *alpha*- and *beta*-peltatin ranged between 2 and 5 micrograms per gram, depending on the vehicle employed. The maximum tolerated dose (MTD) of the three chemicals ranged from 25 to 60 micrograms per gram. The ratio of MTD to MED in mice for a single injection of podophyllotoxin, *alpha*- and *beta*-peltatin was about 10, 20, and 30, respectively.

Different routes of administration, *viz.*, subcutaneous, intravenous, and oral, all produced similar effects. A considerably higher dose level was required for the oral route (about 50 micrograms/gram), and the margin between the MTD and MED was much narrower by this than by the other routes.

Alpha- and *beta*-peltain were soluble in aqueous solutions containing one to two equivalents of sodium hydroxide but had little, if any, solubility in olive oil. Podophyllotoxin was soluble (>5 per cent) in propylene glycol, moderately soluble (0.5 to 1.0 per cent) in olive oil, but only slightly (about 0.01 per cent) in water. All three were active against tumors in all the vehicles listed.

ACTION OF PELTATINS ON LYMPHOMAS AND OTHER TUMORS IN MICE. Ezra M. Greenspan (by invitation), J. Leiter, and M. J. Shear. (Chemotherapy Section, National Cancer Institute, Bethesda 14, Md.)

The effect of subcutaneous injection of alkaline aqueous solutions of *alpha*- and *beta*-peltatin was studied on a variety of intramuscularly transplanted tumors. These included: an acute stem-cell leukemia, a metastasizing lymphosarcoma, a local lymphosarcoma, an adenocarcinoma, and a melanoma, all in mice; and a polymorphic rat carcinoma. A single subcutaneous injection regularly induced extensive gross and microscopic damage in the lymphoid tumors at low dose levels. Maximum tolerated doses were necessary for induction of consistent and extensive cellular damage in the adenocarcinoma, melanoma, and rat carcinoma.

Repeated injection of *alpha*-peltatin in mice bearing lymphoid tumors produced shrinkage of tumors and of metastases, retardation of tumor growth, shrinkage of spleen, and increased survival. *Alpha*-peltatin produced a leucopenia and a delay in infiltration of blast cells in blood and bone marrow of leukemia-bearing mice. Com-

plete regressions were not observed. Multiple low doses were well tolerated in mice bearing established palpable tumors. A large ratio of MTD/MED for a single subcutaneous injection was noted in animals bearing lymphoid tumors. The ratio for both peltatins was in the same range.

Mortality and weight loss, in animals bearing lymphoid tumors, were found to depend on the age and size of the tumors and on the extent of induced tumor damage as well as on the amount of drug administered. Shrinkage and histopathological changes were noted in the thymus, spleen, other lymphoid tissues and testis at maximum tolerated doses.

EFFECT OF A SINGLE INJECTION OF COLCHICINE, COLCHICINE DERIVATIVES AND RELATED COMPOUNDS ON MOUSE TUMORS. V. Downing (by invitation), J. L. Hartwell (by invitation), J. Leiter, and M. J. Shear. (Chemotherapy Section, National Cancer Institute, Bethesda 14, Md.)

Colchicine and ten derivatives, each at its maximum tolerated dose, were injected subcutaneously into mice bearing week-old implants of sarcoma 37. Eight of these derivatives (colchiceine, *N*-benzoyltrimethyl colchicinic acid methyl ether, trimethyl colchicinic acid methyl ether *d*-tartrate, *N*-acetyliodocolchinol and its methyl ether, *N*-acetylcolchinol and its methyl ether, and colchinol) all produced effects similar to colchicine, *viz.*, grossly visible hemorrhage and necrosis, marked mitotic arrests, distorted mitotic figures, and induced generalized pycnosis. (MacCardle and Downing, Cancer Research, **7**:717, 1947; MacCardle, *ibid.*, **8**, 1948. In press.) *N*-benzoyltrimethylcolchicinic acid produced some mitotic arrests but no pycnosis, whereas trimethylcolchicinic acid was entirely negative grossly and microscopically at doses up to 400 micrograms/gram body weight. The MTD of these derivatives was 10 to 450 times that of colchicine; trimethylcolchicinic acid methyl ether *d*-tartrate gave the widest range between the MTD (100 micrograms/gm. body weight) and the minimum effective dose (MED), namely, 2 micrograms/gram of body weight. Seven derivatives were soluble in an aqueous vehicle; 3 were injected as a suspension in oil. The active compounds also damaged other types of mouse tumors, both transplanted and chemically induced.

Approximately 18 phenanthrene derivatives yielded negative results in the same screening procedure, in which the mice were sacrificed at 8, 24, and 48 hours after injection of the compound. Of 163 diphenylethylamines similarly examined, 64 induced damage in sarcoma 37. Microscopically, however, this damage appeared to be different from the effects produced by the colchicine type of compound.

PROPERTIES OF THE PRODUCTS OF HYDROLYSIS OF *SERRATIA MARCESCENS* POLYSACCHARIDES. Hugh J. Creech, Martha W. Wharton (by invitation), Reed F. Hankwitz, Jr. (by invitation), D. R. A. Wharton (by invitation), and Irene C. Diller. (Lankenau Hospital Research Institute and The Institute for Cancer Research, Philadelphia 30, Pa.)

Since it is important clinically to reduce the toxicity and antigenicity of the tumor-necrotizing polysaccharides from *Serratia marcescens*, exploration has been made of the action of glycerophosphatases and other methods of hydrolysis on these polysaccharide-lipid complexes. Preparations of enzymes, obtained by processing rat intestine, were allowed to act upon the complexes at pH 5 and 9. The enzymatic hydrolyses were conducted within a cellophane sac surrounded by suitable buffer solutions which were changed frequently. Tests of the antigenicity, toxicity and tumor-necrotizing activity were made on the total non-dialyzable product at several stages during the hydrolysis. The extent of hydrolysis was determined by suitable micro methods of analysis for glucose and phosphorus.

With an increased degree of hydrolysis, there was a corresponding decrease in the ability of the product to elicit the formation of agglutinins in mice. The products obtained by hydrolysis at pH 5 were more toxic than those obtained at pH 9; a single injection of the products obtained by hydrolysis at pH 5 conferred a greater tolerance on mice toward a subsequently administered lethal injection of unaltered polysaccharide than those obtained under alkaline conditions. Variations occurred among the products obtained at each pH and these could not always be correlated with the degree of hydrolysis. A lack of parallelism was also noted between the extents of tumor-necrotization and hydrolysis. It is anticipated that fractionation of the non-dialyzable products will provide interesting materials for study.

STUDIES ON THE INHIBITION OF SARCOMA 180 IN MICE. C. Chester Stock, Kanematsu Sugiura, Alice E. Moore (by invitation), and C. P. Rhoads. (Division of Experimental Chemotherapy, Sloan-Kettering Institute for Cancer Research, New York, N.Y.)

Inhibition of the development of Sarcoma 180 in mice has been developed as a method of screening materials for adverse effects upon tumor tissue. Twenty-four hours after subcutaneous implantation of Sarcoma 180 into the axillary region of mice, intraperitoneal injections of the chemotherapeutic agent in maximum tolerated doses are initiated and continued twice a day for 7 days. At the end of the injection period the tumors are measured in two diameters by calipers. The inhibition of the tumors in the treated animals is based on the development of the tumors in relation to the untreated controls.

In a study of over 800 compounds the test has eliminated approximately 90 per cent of the materials from further study; yet, it appears sufficiently sensitive to suggest new types of compounds worthy of exploration. The screening test is merely a preliminary to additional studies of therapeutic and cytological effects on Sarcoma 180 and for studies on a spectrum of mouse and rat tumors.

The results obtained with miscellaneous compounds including carbamates, fluorenes, nitrogen mustards, anti-biotics, folic acid analogs, and related compounds are summarized.

EFFECTS OF PODOPHYLLOTOXIN AND PICROPODOPHYLLIN ON BLOOD CELLS AND OTHER TISSUES OF RATS AND MICE. Margaret G. Kelly (by invitation), Ross C. MacCardle, and Paul K. Smith. (Department of Pharmacology, George Washington University Medical School, Washington, D.C., National Cancer Institute, Bethesda, Md., and Department of Pharmacology, George Washington University Medical School, Washington, D.C.)

It was reported last year by this and other laboratories that administration to rats of podophyllin and some podophyllin derivatives produced a transitory leukopenia. The possibility that this effect was mediated through the adrenal glands was investigated by following the changes in the eosinophiles and by histologic study of the adrenal glands.

Counts were made at one and twenty-four hours after single intraperitoneal injection of 1 and 10 mg. per kilo of podophyllotoxin and 7 and 75 mg. per kilo of picropodophyllin. A significant decrease in the number of eosinophiles in the circulating peripheral blood was found and was accompanied by depletion of the osmiophilic granules in the adrenal glands.

Swiss mice bearing 6-day old intramuscular implants of Sarcoma 37 in the right thigh received a single intraperitoneal injection of either podophyllotoxin (10 mg. per kilo) or picropodophyllin (75 mg. per kilo). The mice were killed at intervals ranging from 8 hours to 10 days after injection and no significant change was observed in histologic preparations of liver, spleen, lung, kidney, and intestine. The tumor tissue, however, showed necrosis and mitotic arrests and the osmiophilic granules were greatly depleted in the fasciculata of the adrenal gland. Chronic toxicity changes in tissues are being studied in normal Swiss mice.

GLYCOLYTIC INHIBITOR THERAPY IN HUMAN MALIGNANT NEOPLASIA. Maurice M. Black, Israel S. Kleiner (by invitation), and Herman Bolker (by invitation). (Department of Biochemistry, New York Medical College, New York, N.Y.)

In vitro metabolic studies of tumor tissue reveal a striking uniformity among diverse tumor types in regard to accentuation of aerobic and anaerobic glycolysis. The use of the glycolytic inhibitors fluoride, iodoacetate, and malonate on a series of more than 100 cases of diverse forms of human cancer was accompanied by objective evidence of tumor inhibition in cases of acute leukemia, Hodgkin's Disease and lymphosarcoma. This was not the case in patients with carcinoma of the colon, fundus uteri, ovary, pancreas, rectum, or in chronic leukemia, melanoma, chorioepithelioma testis, or with squamous cell cancers of the cheek and pharynx. Partial or questionable therapeutic response occurred in cancers of the breast, adrenal cortex, cervix, lung, stomach, testes, and in fibrosarcoma. These observations suggest that while certain cancer types behave as if they were particularly dependent on glycolytic mechanisms for their energy requirements, this is certainly not the case for all or even a majority of them.

The limitation of the therapeutic efficacy of these agents to individual tumors or related neoplastic types may be explained by either of two possible alternatives. Either the inhibitory effect with these compounds represents an action on a unique metabolic or structural feature of these growths, or the concept of an essential entity of all malignant neoplasia as tacitly predicated on the basis of chemical and cytological observations is false or inadequate. The *in vitro* biochemical characterization of tumor tissue may be a meaure of a manifestation of malignancy rather than of its subtle essence or it may fail to reflect the ability of tissues to adapt to an unfavorable environment by alterations of their metabolic pathways.

A STUDY OF N-IODOACETYL AMINO ACIDS IN RELATION TO INHIBITION OF TUMOR GROWTH. Orrie M. Friedman and Alexander M. Rutenburg. (Introduced by A. Seligman.) (Department of Chemistry, Harvard University, Cambridge, Kirstein Laboratory of Surgical Research, Beth Israel Hospital, Boston, and Department of Surgery, Harvard Medical School, Boston, Mass.)

Since toxic substances related to essential metabolites seemed of interest for a study of inhibition of growth of tumors, derivatives of amino acids were prepared which were toxic and which could be readily labelled with a radioactive isotope. N-iodoacetyl derivatives of tryptophane, leucine and phenylalanine have been prepared. The relative toxicity in Swiss mice of these three substances and iodoacetamide was determined and the ability of the four substances to inhibit the growth of Sarcoma 37 in this test animal was studied. The results have indicated that these substances inhibit the growth of this tumor significantly, to different extents and in a manner apparently unrelated to systemic toxicity.

The radioactive analogues of the three iodoacetyl amino acids and iodoacetamide have been prepared by the use of I^{131} The concentration of radio-activity and its disappearance from blood, tumor and liver following intravenous injection of these substances have been determined. Radioactivity has been found in the three tissues in significant amounts, the concentration of activity in tumor being consistently greater than in liver and less than in blood. The rate of disappearance of radioactivity from tumor in the case of the three amino acid derivatives followed a similar characteristic pattern different from that of iodoacetamide.

INTRA-CYTOPLASMIC PHYSIOLOGICAL MEDIA. M. J. Kopac. (Department of Biology, Washington Square College of Arts and Science, New York University, New York 3, N.Y.)

Cell inclusions and submicroscopic particulates isolated by centrifugal fractionation especially for enzymic determinations require media that maintain such cytoplasmic fractions in essentially native states. Such media are being developed and evaluated by methods involving microinjection and micro-surface chemical techniques. Experimental media that produce no appreciable

disturbances in the cytoplasm on microinjection, nor changes in structure and properties of mitochondria, microsomes, and nuclei, streaming, or sol-gel transformations, should preserve formed components, on disintegration of the cell, in reasonably native states for subsequent isolation.

Thus far, the best medium for Myxomycete and ameba cytoplasm contains KCl, $CaCl_2$, and NaCl, in which K/Na = 9, and (K + Na)/Ca = 40. A satisfactory medium for isolating Arbacia egg inclusions contains KCl and Na-citrate since ionic Ca must be avoided. For micromanipulative studies on salivary gland chromosomes, the medium must be K-rich and Ca-free.

Previous work has shown that cytoplasmic proteins in intact cytoplasm do not become surface denatured at oil/water interfaces. Accordingly, intracytoplasmic media were tested by surface chemical methods that can measure changes in surface denaturation of proteins at oil/water interfaces established in living cells. By simultaneously injecting aqueous media with the indicator oil, the action of these media can be compared with that produced on introduction of the oil/water interface alone (traumatic cytolytic effect).

Preliminary measurements indicate that media otherwise satisfactory for ameba cytoplasm significantly increase the surface denaturation of cytoplasmic proteins of the ameba. Intra-cytoplasmic media, therefore, should be further modified by adding calcium binders, chelating agents, nucleic acids, or anti-surface denaturating agents exemplified by *bis*-amidinomethyldibenzyl or protamine.

K-rich, Na-poor solutions fortified with either nucleic acids or *bis*-amidinomethyldibenzyl produce much less surface denaturation of cytoplasmic proteins. None, however, produces the low degree of surface denaturation obtained at oil/water interfaces established without previous injection of intracytoplasmic media. Continued investigations should yield better media for isolating complete enzyme systems in the form, probably, of submicroscopic cytoplasmic particulates.

POLAROGRAPHIC STUDIES ON THE LIPIDS OF EPIDERMIS UNDERGOING CARCINOGENESIS WITH METHYLCHOLANTHRENE. C. Carruthers and V. Suntzeff. (Department of Anatomy, Division of Cancer Research, Washington University Medical School, St. Louis, Mo.)

An alteration in a lipid occurs during the process of epidermal carcinogenesis in mice. Polarography of the lipid extractable material of mouse epidermis undergoing carcinogenesis was carried out in unbuffered and buffered mixtures of water, dioxane (50 per cent by volume) and tetrabutylammonium iodide as supporting electrolyte. The lipids of normal and hyperplastic epidermis gave a double or two polarographic waves while induced or transplantable squamous cell carcinomas showed only a single wave. In buffered solutions the waves of the lipids of normal and hyperplastic epidermis were found to be pH dependent. The single wave of the lipids of the carcinoma disappeared in buffered solutions showing that its presence in the unbuffered mixture was due to some lipid substance which acted as a buffer.

The lipid material of the carcinomas did have a very small amount of a reducible lipid, the half-wave potential of which was significantly more positive than that of the first wave of epidermis, and is probably a compound characteristic of the carcinoma. This information indicates that epidermis in becoming carcinomatous either loses the lipid having the double polarographic wave or that this lipid is qualitatively altered from that found in the carcinomas. Normal human epidermis has the same double polarographic wave as the mouse, and the process of carcinogenesis in both species seems to be similar with respect to these reducible lipids. Efforts are now in progress to ascertain the nature of the reducible lipids.

HISTOCHEMICAL STUDIES OF MOUSE HEPATOMAS PRODUCED BY CARBON TETRACHLORIDE. C. S. Lee (by invitation), R. E. Stowell, and A. Villisana (by invitation). (Departments of Oncology and Pathology, University of Kansas Medical School, and Department of Pathology, Washington University School of Medicine, St. Louis, Mo.)

The histochemical and cytological changes in the livers of mice following single and repeated feedings with carbon tetrachloride were followed through the stages of cirrhosis and hepatoma formation. At monthly intervals, 53 experimental and 15 control animals were started on the diet and all animals were killed and tissues fixed at one time. In addition to the usual trabecular hepatomas, growth with acinous and lymphangiomatous nature were seen.

Tissues were stained for alkaline phosphatase, glycogen, lipase, ribose nucleic acid, desoxyribose nucleic acid, lipids, bile ducts, and mitochondria. As compared with normal liver tissue cirrhotic liver showed only slight changes in alkaline phosphatase, glycogen and lipase reaction, while most of the hepatoma cells showed stronger staining reactions for alkaline phosphatase, glycogen, and reduced lipase reaction. Ribose nucleic acid was increased in some hepatomas. The differences in the histochemical staining reactions between the cirrhotic and hepatoma tissue were frequently pronounced.

After a single feeding of carbon tetrachloride the initial necrosis was followed by increased mitotic activity and regeneration. Alkaline phosphatase and glycogen decreased markedly the first day and then gradually increased especially in the central parts of the lobule. Lipase activity decreased through the third day and started to increase five days after treatment.

CYTOCHEMICAL STUDIES OF NUCLEOPROTEINS IN NUCLEI OF A TRANSPLANTED TUMOR (SARCOMA 180). Cecilie Leuchtenberger. (Department of Zoology, Columbia University, New York, N.Y.)

The nucleoprotein content of resting nuclei of a viable tumor tissue was compared with that of pycnotic nuclei of necrotic areas of the same tumor. The relative amounts of desoxyribosenucleic acid (from the Feulgen reaction) and protein (from the Millon reaction) in the

different nuclear types were estimated in fixed sections by the microscopic photometric method of Pollister and Ris. The methylgreen staining was used to detect a change in the physical state of the nucleic acid. Cytologically 4 different types of tumor cells were selected for this study: a viable tumor cell (characterized by a distinctly basophilic cytoplasm, a spherical nucleus, and a large nucleolus) and three progressive stages of pycnosis as judged by the increasing amount of condensation of the chromatin and reduction of the nuclear volume. The earliest pycnotic stage is characterized by a rounding up of the nuclei, by a marked reduction of the nuclear volume, by a decrease in the protein content, and a considerable reduction of the affinity of the chromatin for methylgreen. The total desoxyribosenucleic acid, as indicated by the Feulgen stain, remains practically unchanged in comparison with the nuclei of the viable tumor cells. The loss of the stainability with methylgreen suggests that depolymerisation of the desoxyribosenucleic acid has taken place. Later pycnotic stages show a further decrease in the nuclear volume and protein content and a partial loss of the desoxyribose nucleic acid. Whereas, in pycnosis, the reduction in the nuclear volume and protein content and the nucleic acid depolymerisation are extensive at an early stage, the actual decrease in the amount of desoxyribosenucleic acid does not become pronounced until much later.

PHASE MICROSCOPE STUDIES OF MALIGNANT CELLS. Robert P. Zanes, Jr. (by invitation), Dorothy Eshbaugh (by invitation), and Herman A. Hoster. (Ohio State University College of Medicine, Columbus, Ohio)

Scraping of the cervix and aspiration from the vaginal canal and cervix at the time of pelvic examination have been used extensively in attempting to establish or rule out the presence of neoplastic disease using the Papanicoloau technique. This report is a preliminary discussion of observations made on material obtained from the same source and studied by means of the phase contrast microscope using unstained and supravitally stained smears. The specimens were studied within two hours after preparation in order to confine observation to cells in the living state. An attempt has been made to correlate these observations with the clinical findings, the histo-pathologic pattern, Papanicoloau smear interpretations, and changes due to recent radiation therapy.

In two hundred cases of normal individuals and individuals with biopsy-proven neoplasia, the cyto-morphologic criteria suggesting malignancy appeared to be: (a) an increase in nuclear size and irregularities in nuclear shape; (b) the presence of multiple, assymetrical nuclei; (c) the presence of large, irregular and multiple nucleoli; and (d) the presence of refractile cytoplasmic bodies, sometimes identical in size and shape and occasionally varying markedly. These bodies are observed arranged in a ring around the nucleus or in a rosette formation in a hof of the nucleus.

Preliminary comparisons with the results of the Papanicoloau examination reveal agreement in approximately 70 per cent of the cases studied. No statement can be made at this time concerning an increased efficiency of the phase contrast smear technique as compared with that of the Papanicoloau technique. The phase technique has the advantage of providing supplementary diagnostic information at the time of the pelvic examination without the delay associated with fixation and staining.

STUDIES WITH A RADIOACTIVE IODOTETRAZOLIUM COMPOUND AND WITH A NEW TETRAZOLIUM SALT WHICH YIELDS A BLUE PIGMENT ON REDUCTION. Arnold M. Seligman, Raplh Gofstein (by invitation), and Alexander M. Rutenburg (by invitation). (Department of Surgery, Beth Israel Hospital, Boston, and Harvard Medical School, Boston, Mass.)

Diphenyl (*p*-iodophenyl) tetrazolium chloride has been prepared with radioactive iodine (I^{131}) in a series of synthetic steps from 1 mg. of aniline and benzal phenyl hydrazone in a single reaction vessel. Following intravenous injection of the radioactive tetrazolium salt into tumor bearing mice, radioactivity in the circulating blood dropped in $\frac{1}{2}$ hour to 2 to 4 per cent of that injected. The highest concentrations of radioactivity were found in kidney, liver, and lung. Radioactivity persisted at higher levels in lung and in mesenteric fat. Sarcoma 37 contained less radioactivity than most other tissues.

A water soluble, pale yellow tetrazolium salt has been prepared in good yield from the formazan obtained from benzal phenyl hydrazone and diazotized dianisidine. The formazan produced by enzymatic or chemical reduction of the tetrazolium compound is a deep blue water insoluble pigment. This pigment when dissolved in organic solvents may be measured colorimetrically in concentrations less than 0.5 microgram per cc. The tetrazolium compound is about as toxic as triphenyl tetrazolium chloride. It is being used in the development of histochemical methods for demonstrating oxidation-reduction systems in normal and neoplastic tissues.

NEW METHODS FOR THE HISTOCHEMICAL DEMONSTRATION OF INTRACELLULAR ENZYMES. Arnold M. Seligman, and Leon H. Manheimer, and Marvin Nachlas (by invitation). (Department of Surgery, Beth Israel Hospital, Boston, and Harvard Medical School, Boston, Mass.)

Utilizing the method first developed by Menten *et al.* for alkaline phosphatase, substrates have been prepared from naphthols which are split by intracellular enzymes in the presence of stabilized diazonium salts. Immediate coupling, which results with the naphthols as they are released, produces insoluble colored pigments in tissue sections at the site of the enzymatic activity. The following enzymes can be demonstrated: alkaline phosphatase, three varieties of acid phosphatase, non-specific esterase, lipase, sulfatase, and *B*-blucuronidase.

COLOR REACTIONS WITH MALIGNANT SERA. Emil Weiss. (Department of Pathology, Peoples Hospital, Chicago 16, Ill.)

A number of aniline dyes in dilutions 1:10.000 do not show any color changes on addition of malignant sera. Sera of normal individuals, those afflicted with various diseases or benign tumors turn the original blue color of the same dyes green. The glassware must be clean and free of any acid or dye. The sera must be fresh and thoroughly centrifuged. Turbid, hemolyzed, or icteric sera can not be used. The dyes are kept in 0.1 per cent stock solutions of rubbing alcohol (70 per cent isopropyl alcohol). The stock solution is diluted with distilled water 1:10 before use. To 0.5 cc. of the diluted stock solution of the dye 0.5 cc. of serum is added, the tube is shaken for a few seconds and the color changes are noted. A positive control containing a known malignant serum and a negative control containing a known normal serum are handled in the same manner. A dye control correspondingly diluted with water also is used. The tubes are placed overnight or for 12 hours in the refrigerator and then the final reading is made. Eventual color changes become more distinct after standing several hours. Over 300 sera were examined with the above technic. Positive (blue) reactions were obtained in 89.1 per cent of malignant sera. Azur II, toluidine blue, trypan blue, and victoria blue were found to be equally useful. This method applies to all types of malignancy. Hodgkin's disease and various forms of leucemia give consistently positive results.

FURTHER STUDIES ON THE RELATIONSHIP OF MUCOLYTIC ENZYMES TO THE INVASIVE GROWTH OF CARCINOMAS AND SARCOMAS IN MICE. William L. Simpson, A. R. T. Denues (by invitation), and Patricia J. Keller (by invitation). (Detroit Institute of Cancer Research, Detroit 1, Mich.)

The capacity for invasive growth forms the most critical point of differentiation of malignant cells from normal cells. In previous studies by Simpson *et al.*, enhancement of this property has been associated with injections of the spreading factor from testes, hyaluronidase. Coman and his collaborators failed to find such an effect with a benign tumor and with a transplantable sarcoma. Additional series of mouse transplantable squamous cell carcinomas and fibrosarcomas have been analyzed for the effects of local injections of hyaluronidase on invasive growth and metastasis. The findings in these experiments will be presented in detail.

SEVERAL FACTORS THAT INFLUENCE *p*-DIMETHYLAMINOAZOBENZENE CARCINOGENESIS IN THE RAT. Paul N. Harris. (Lilly Research Laboratories, Eli Lilly and Co., Indianapolis, Ind.)

Purified diets containing .09 per cent of *p*-dimethylaminoazobenzene. were administered continuously in these experiments until the rats died or were killed for microscopic examination of their tumors. The rapidity of tumor development was influenced by the strain of rat employed. There was no difference between the Evans, Sprague-Dawley, and Harlan strains (the Haran strain, obtained from a local breeder, is descended from the Wistar strain). As compared with these strains, tumor development in rats obtained from the Wistar Institute was somewhat retarded, and in the Carworth Farms strain was even more delayed. Variation in the amount of cottonseed oil (5, 10, and 20 per cent) in the diet had no effect upon carcinogenesis. With a diet containing 20 per cent of olive oil, tumor development was slower than with diets containing 5 per cent and 10 per cent. A diet containing 5 per cent of corn oil gave less rapid tumor development than diets containing 10 per cent and 20 per cent. With a low level of riboflavin (2 mg./kg.), there was no difference in tumor development in rats given 10 per cent and 30 per cent casein diets, but with a riboflavin level of 100 mg./kg., there was great retardation of carcinogenesis with a 10 per cent casein diet. With a constant level of riboflavin (7.2 mg./kg.) and pyridoxine (3 mg./kg.), a diet containing 10 per cent of casein gave less rapid tumor development than did one containing 7 per cent of casein and 3 per cent of liver extract.

ENZYME INHIBITION IN RELATION TO CHEMOTHERAPY. W. W. Ackermann (by invitation), and V. R. Potter. (McArdle Memorial Laboratory, University of Wisconsin, Madison 6, Wis.)

Studies are presented which emphasize the importance of the dissociation constant of the enzyme-inhibitor complex in relation to enzyme concentration. When the constant is sufficiently small the per cent inhibition of the enzyme will be determined not only by the concentration of the inhibitor but also by the concentration of the enzyme. By means of a graphic method, in which the rate of reaction was plotted against enzyme concentration, it was possible to show that in the case of certain inhibitors having very small dissociation constants the activity of the enzyme was approximately proportional to (E)-(EI), where (E) was the total concentration of enzyme and (EI) was approximately the concentration of added inhibitor. To describe the relationship a general equation was developed to express the concentration of the enzyme-substrate complex in terms of the total amount of enzyme, the amounts of substrate and inhibitor and the dissociation constants of the enzyme-substrate and enzyme-inhibitor complex.

From the standpoint of chemotherapy, these studies lead to the suggestion that it may be possible to inactivate specifically an enzyme in cancer tissue even though the enzyme is not unique to cancer tissue. If a highly specific inhibitor with a low dissociation constant is used, and if the enzyme is present in lower concentrations in cancer tissue than in normal tissues, it should be possible to inactivate all of the enzyme in the cancer while inactivating smaller percentages of the enzyme in normal tissues.

PRODUCTION OF PROFOUND CHANGES IN BACTERIA BY RHYTHMIC EXPOSURE TO HEAT. R. R. Spencer and M. B. Melroy. (National Cancer Institute, Bethesda, Md.)

Heat has been shown to be both a carcinogen and a mutagen (Demerec. Brit. J. Cancer, 2: 114–117, 1948).

By the use of high temperatures, a technique has been developed which results in profound permanent alteration of bacteria. The fundamental principle underlying this technique is a rhythmic injury-repair cycle of exposure extending over a long period of active multiplication of the species propagated in serial cultures.

Analysis of the experimental data reveals the importance of three rhythmic factors in the adaptation of bacterial species to an unfavorable environment (high temperatures). These are: a) *The temperature rhythm* (the duration of the interval of exposure); b) *The rest rhythm* (the duration of the period of freedom from heat); c) *The transfer rhythm* (the interval of transfer of the serial cultures).

These rhythms and their interrelationships determine to a large extent the survival and adaptation of the species or its failure to survive. It is suggested that carcinogenesis may be basically a problem in survival and adaptation of somatic cells to unfavorable environments over long periods of time.

A HISTOPATHOLOGIC AND GENETIC STUDY OF TUMORS OF THE FORESTOMACH IN MICE TREATED WITH A SINGLE SUBCUTANEOUS INJECTION OF METHYLCHOLANTHRENE.

M. A. Bagshaw (by invitation), and L. C. Strong. (Department of Anatomy, Yale University School of Medicine, New Haven, Conn.)

Approximately 3,000 mice of the pBr subline were injected subcutaneously with 1.0 mg. of methylcholanthrene at 60 days of age. Local lesions developing at the site of injection were previously described. An analysis of the first 78 tumors of the forestomach is presented. The mean latent period was 454 days.

Hematoxylin-eosin, Laidlaw's silver reticulum, Masson, and Van Gieson's stains were employed. Ninety-nine per cent of the tumors were classified as either differentiated epidermoid carcinomas, spindle cell neoplasms, or mixed tumors. One adenocarcinoma and one round cell tumor were found. The differentiated epidermoid carcinomas were composed of sheets of epithelial cells containing basal cells, spinous cells, and keratinized elements. The spindle cell neoplasms were characterized by bands and whorls of spindle shaped cells which contained scanty, ill defined cytoplasm and long, oval, usually hyperchromatic nuclei. The mixed tumors contained epidermoid as well as spindle cells. Comparison of the above three types revealed numerous characteristics in common. The term, sarcoma, for the spindle cell lesion seemed unjustified. Metastases throughout the abdomen and thorax occurred frequently. Transplantation of the epidermoid carcinoma to homologous hosts was successful. Intraperitoneal transplants resulted in tumors which killed the new hosts within 2 or 3 weeks and were morphologically identical to the spindle cell neoplasms.

Genetic analysis revealed relatively more stomach tumors, irrespective of type, in a single lineage over a period of 5 generations.

RELATIONSHIP BETWEEN THE HAIRLESS GENE AND SUSCEPTIBILITY TO INDUCED PULMONARY TUMORS IN MICE.

W. E. Heston and Margaret K. Deringer. (National Cancer Institute, Bethesda, Md.)

High-pulmonary-tumor strain A mice were outcrossed to a strain of pink-eyed hairless mice and the F_1 progeny in turn backcrossed to the pink-eyed hairless strain. This backcross generation provided a test for linkage between susceptibility to pulmonary tumors and both the pink-eye gene (*p*) and the hairless gene (*hr*). Ninety-two of these backcross mice were injected intravenously each with 0.5 mg. dba dispersed in 0.5 cc. horse serum at from 2 to 3 months of age and were killed 5 months later and the number of pulmonary tumors in each was recorded. No linkage between susceptibility to pulmonary tumors and the pink-eye gene was evident since the proportion of pink-eyed segregants with pulmonary tumors and the mean number of nodules found in the pink-eyed segregants was not significantly different from that of the non-pink-eyed segregants. There was, however, a relationship between susceptibility to the induced pulmonary tumors and the hairless locus. The hairless segregants were less susceptible. When the hairless mice were compared with haired in regard to proportion with tumors, the difference was borderline in significance but when compared in regard to average number of nodules per individual, a more delicate measure of susceptibility, the difference was highly significant statistically. Growth curves of non-injected backcross mice indicated no difference between pink-eyed and non-pink-eyed segregants, but the hairless segregants were smaller than the haired. Possible relationship between the effect of the hairless gene on growth and its effect on susceptibility to pulmonary tumors is discussed.

A "MATERNAL INFLUENCE" ON THE GROWTH OF A TRANSPLANTABLE TUMOR IN MICE.

Morris K. Barrett and Walter C. Morgan (by invitation). (National Cancer Institute, Bethesda 14, Md.)

That the incidence of spontaneous tumors and the successful inoculation of transplantable tumors are strongly influenced by genetic background has been long established. It is also known that there is a "maternal influence" on the incidence of certain spontaneous tumors and the "take" of certain transplantable tumors.

A transplantable mammary carcinoma which originated in a C3H mouse was inoculated into F_1 hybrid mice derived from reciprocal crosses between strain C3H and strain C (B alb C). The tumor grew progressively in all mice but a difference in the rate of growth was observed that depended upon which strain was the maternal line of the hybrids and which the paternal. In all of eleven experiments the average weight attained in three weeks by the tumors was greater in the case of tumors growing in hybrids of the C3H maternal line. Under the conditions of these experiments the maternal influence noted was independent of the sex and age of the hosts, the absolute size of the tumors, the presence of

intercurrent disease, the donor of the graft (whether inbred or hybrid) and annual variations in laboratory conditions. The nature of the factor or factors which account for this maternal influence was not disclosed by these experiments.

THE EFFECT OF CROWDING ON THE PENETRANCE OF AN HEREDITARY MELANOMA OF DROSOPHILA MELANOGASTER.* M. H. HARNLY (by invitation), E. D. GOLDSMITH, and F. FRIEDMAN (by invitation). (Washington Square College and the College of Dentistry, New York University, New York, N.Y.)

Crowding has been found to affect the penetrance of a benign hereditary melanoma in Drosophila melanogaster. Single pair matings in 1×4 inch vials gave a significant difference in penetrance of the tumor for one and eight day egg laying periods. To determine the effect of known intensity of crowding (developing animals per vial) on the percentage of adult animals having this melanotic tumor, groups varying from 5 to 200 just hatched larvae were placed in vials of food. Approximately 85 per cent of the adults had tumors at all concentrations and 400 larvae per vial has given a similar value. When the longer egg laying period was simulated by adding 100 larvae per day for four days in the vials (a total of 400 larvae per vial) the penetrance dropped from 85 per cent to 54 per cent on the large number of animals tested. Obviously, crowding as such does not affect the penetrance of this hereditary melanoma. The data suggest that the critical time in development for the onset of tumor growth is during the first larval instar and environmental changes resulting from crowding determine in large part the penetrance or non-penetrance of this melanoma. These points are now under investigation.

ADRENAL CORTICAL TUMORS IN A RECIPROCAL CROSS. GEORGE W. WOOLLEY and MARGARET M. DICKIE (by invitation). (Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine)

A cross was made reciprocally between two strains of mice to see if maternal influences were of importance in the inheritance of adrenal cortical tumors. The results indicate that there is no major maternal influence, such as has been found with mammary tumors, associated with the inheritance. The tumors were of the type obtained following gonadectomy. Some observations on tumor development are included.

AGE AND LYMPHOMA INCIDENCE IN CF-1 MICE. AUSTIN M. BRUES, MIRIAM P. FINKEL, HERMANN LISCO, and GEORGE A. SACHER (by invitation). (Argonne National Laboratory, Chicago, Ill.)

Three large groups of CF-1 female mice, a total of 2500 animals, have been maintained throughout life as controls for radiation experiments. The median life span of these animals is about 600 days and about 1 per cent survive beyond 850 days.

* Aided by a grant from The National Cancer Institute, National Institute of Health, U.S. Public Health Service.

Throughout most of life, the susceptibility to lymphoma and lymphatic leukemia is approximately doubled at intervals of 85 days. After 700 days of age no further increase in morbidity rate is observed. This pattern of incidence is similar to that seen in the case of many human tumors. The three groups of mice were obtained over a three-year period, and the age incidence of lymphoma has remained nearly constant.

MONOZYGOTIC TWINS WITH SIMILAR BREAST PATHOLOGY SUGGESTING THE ORIGIN OF MALIGNANT FROM BENIGN NEOPLASIA. DUDLEY JACKSON, JR. (by invitation), DUDLEY JACKSON, SR. (by invitation), F. W. STEINBERG by invitation), and C. P. OLIVER. (Nix Memorial Hospital, San Antonio, and University of Texas, Austin, Texas)

Twins "A" and "B" have strikingly similar breast pathology. Cysts were removed from "A's" right breast at age 28. When the twins were age 36, "A" had a bleeding left nipple, and a simple mastectomy was performed. "B" had cysts removed from both breasts and "A" had a cyst removed from her right breast in 1941. "A's" tissue showed "epithelial hyperplasia." "B" had another cyst excised a year later, and this also had "epithelial hyperplasia." "B" developed the next tumor July, 1945, and the pathologist reported "chronic cystic mastitis, multiple papillary cystadenomas and intracanalicular adenofibroma and cysts with marked papillary infoldings." In October, 1945, "A's" right breast showed epithelial hyperplasia and pathological features very similar to "B's" report of three months earlier. "B" had a lump in her left breast in October, but delayed surgery. Simple mastectomy was done on "A's" right breast in December at the request of the pathologist who considered the lesions found on the biopsy in October to be "precancerous." Twelve days later, biopsy of "B's" left breast showed intraductal adenocarcinoma and radical mastectomy was done. Histological examination of the tissue was suggestive of a transition from papillary epithelial hyperplasia to malignancy. The twins' monozygotic origin was determined from studies of the AB, MN, and blood types, PTC taste thresholds, dermatoglyphics, and other physical characteristics. The family history shows cancer, glandular deficiency and diabetes among the twins' relatives. The twins' parents and other relatives were short lived. The twins and some relatives are highly infertile.

COMPLEMENT FIXATION IN ANIMAL NEOPLASIA. II. DEVELOPMENT OF THE REACTION IN NEW ZEALAND RABBITS CARRYING THE BROWN-PEARCE CARCINOMA. LESTER D. ELLERBROOK, MARK RHEES, AND HELEN THORNTON (by invitation), and STUART W. LIPPINCOTT. (Department of Pathology, School of Medicine, University of Washington and the Cancer Control Division, National Cancer Institute)

The inoculation of the neoplasm which had been carried in New Zealand white rabbits into adult animals of the same breed resulted in the production of definitely

positive reactions in the majority of the animals developing tumors. Tests of sera obtained by serial bleedings of these animals as a rule showed definite complement fixation at 2 to 3 weeks after inoculation and the degree of fixation tended to increase with the continued development of the neoplasm.

The maximum reactions were obtained with sera inactivated at approximately 60° C. Titers were calculated from the volumes of complement required to reach the end-point of 50 per cent hemolysis in the presence of normal serum and of test serum, both alone and in the presence of antigen. Thus far definitely positive reactions were not obtained with rabbits bled serially or with those inoculated with such materials as turpentine, human serum, or normal rabbit muscle.

NATURAL AND IMMUNE ANTIBODIES IN MICE WITH LOW AND HIGH TUMOR INCIDENCE. Israel Davidsohn (by invitation), and Kurt Stern. (Mount Sinai Medical Research Foundation and the Chicago Medical School, Chicago, Ill.)

Natural heteroagglutinins for sheep red cells and for human red cells were investigated in six inbred mouse strains: C57 black, Bagg albino C, C3H, dba, Marsh-albino, and Akm. Striking differences were found in the ability of the serum of these strains to agglutinate sheep red cells. Natural antisheep agglutinins were absent in 40 to 60 per cent of the serum of mice of the C3H, dba, Marsh-Albino, Bagg albino C and Akm strains; the agglutinins present in these strains showed low titers (less than 16). In contrast, animals of the C57 black strain showed presence of antisheep agglutinins in more than 90 per cent, with titers above 16 in more than 50 per cent. No such strain differences were found as to presence and titers of natural agglutinins for human red cells.

Determinations of antisheep agglutinins and hemolysins, following intraperitoneal injection of sheep red cells into C57 black, dba, and C3H animals, revealed a higher antibody production in the C57 black mice than in the two other strains. Similar results were obtained in the three strains in regard to antibodies produced by injection of human red cells.

The significance of these findings is discussed in the light of previously reported differences in the storing ability of these strains. Both observations may be related to differences in the riticulo-endothelial activity of the various strains.

STRUCTURAL AND FUNCTIONAL CHARACTERISTICS OF SPONTANEOUS THYROID TUMORS IN A SWORDTAIL FISH SPECIES.* Aubrey Gorbman (by invitation), and Myron Gordon. (Barnard College, Columbia University and New York Aquarium, New York Zoological Society, New York, N.Y.)

Tumorous enlargement and metaplasia of the thyroid has been observed in a relatively high incidence in older specimens of both sexes of swordtail, *Xiphophorus montezumae*. Thyroidal tumors occur only rarely under similar laboratory and dietary conditions in related species: *Xiphophorus pygmaeus*, *Platypoecilus maculatus*, and *Platypoecilus xiphidium*. They have not been observed in *X. hellerii*, *P. variatus*, or *P. couchianus*. The diet for all species consists of fresh liver, Pablum cereal, live tubificid worms, and dried ocean shrimp. In nature *X. montezumae*, *X. pygmaeus*, and *P. variatus* are found living together occasionally in the Rio Axtla, San Luis Potosi, Mexico. *X. montezumae*'s greater susceptibility may indicate a specific genetic difference.

The main mass of the tumors in *X. montezumae* varying from 35 to 50 millimeters in length is about 5 mm. long and 4 mm. dorsoventrally. Histologically the tumor appears to consist mostly of a microfollicular and afollicular mass, well vascularized, and with abundant stroma. At the edge of the growth a few larger colloid-containing follicles may be found. Follicles may be found within bones and muscle, and heavily invading the gills, obviously interfering with respiratory function.

Administration of tracer radioiodine to tumorous animals and subsequent radioautography reveals that the bulk of the tumor takes up no iodine. Almost normal iodine intake is exhibited by the few peripheral colloid-filled follicles.

THE EFFECT OF A PTEROYLGLUTAMIC ACID ANTAGONIST ON THE RESPONSE OF THE AMPHIBIAN IMMATURE OVIDUCT TO ESTROGEN.* E. D. Goldsmith, Sidney S. Schreiber (by invitation), and Ross F. Nigrelli. (Department of Histology, New York University School of Dentistry and New York Aquarium, New York Zoological Society, New York, N.Y.)

Newly metamorphosed frogs (*Rana clamitans*) were treated parenterally with estradiol benzoate, pteroylglutamic acid (PGA), and 4-amino-pteroylglutamic acid (aminopterin). One group of animals received several dosage levels of aminopterin, or PGA, or both, and was simultaneously treated with the estrogen. A second group was pre-treated with aminopterin, PGA, or both, for 2 to 3 weeks, and then, in addition, was injected with estrogen for a period of 2 weeks. Grossly, the oviducts of the estradiol treated controls exhibited marked enlargement and coiling, whereas, the oviducts of the animals which received estradiol and aminopterin resulted in only slight enlargement and no coiling. Injections of estradiol for 2 weeks in animals pre-treated with PGA were followed by oviduct growth greater than that observed in estradiol controls. PGA in ratios of 100: 1 of PGA to aminopterin showed but slight reversal of the antagonist effect. Histological observations confirmed the gross findings. Mitotic counts and their possible significance as to the site of action of the PGA antagonist in inhibiting growth in the presence of growth stimulating factor(s) will be discussed.

STUDIES ON THE MECHANISM OF ACTION OF AMINOPTERIN (4-AMINOPTERYLGLUTAMIC ACID) ON THE LYMPHATIC TISSUES

* Work aided by a grant from National Cancer Institute, U.S. Public Health Service.

* Aided by a grant from the National Cancer Institute, National Institute of Health, U.S. Public Health Service.

OF MICE. J. H. Dougherty (by invitation), and T. F. Dougherty. (Division of Oncology, Departments of Pathology and Anatomy, University of Utah College of Medicine, Salt Lake City, Utah)

Aminopterin (4-amino-pteryl glutamic acid) is one of a group of substances which produces acute involution of lymphatic tissues. This compound, a folic acid antagonist, has received attention as a chemotherapeutic agent in acute leukemia. Since many agents produce lymphatic tissue involution through pituitary-adrenal cortical mediation, it was of interest to ascertain whether or not aminopterin acted through this mechanism.

Aminopterin in saline was administered intraperitoneally in dosages of 0.02 mg. daily to adrenalectomized and unoperated CBA mice. This amount is lethal for mice in approximately one week. After 5 days of treatment the animals were sacrificed and lymphatic tissues studied. A relative and absolute decrease in size of all lymphatic tissues was observed in the unoperated treated animals when these were compared with control mice of the same age. Involution of lymphatic tissues was not found in the adrenalectomized group although these animals lost as much weight as did the unoperated mice. A progressive absolute lymphopenia was observed in the unoperated animals after one day of treatment. A slight lymphopenia occurred in adrenalectomized animals after 5 days of treatment and there was an inhibition of the lymphocytosis characteristic of adrenalectomized mice. This amount of aminopterin failed to inhibit the growth of subcutaneous transplants of the Gardner lymphosarcoma (C3HED) although it produced acute involution of the lymphatic tissues of the tumor-bearing mice.

It is concluded that much of the effect of large doses of aminopterin on lymphatic tissues of mice is mediated through the adrenal cortex although there is evidence of some direct inhibitory action.

THE EFFECTS OF 4-AMINO-N^{10}-METHYL-PTEROYL GLUTAMIC ACID AND 2,6-DIAMINOPURINE ON THE LEUKOCYTES OF THE NORMAL AND LEUKEMIC MOUSE.* J. H. Burchenal,† J. L. Beidler, and J. Nutting (Introduced by C. P. Rhoads). (Section on Mouse Leukemia of the Division of Experimental Chemotherapy, of the Sloan-Kettering Institute for Cancer Research, New York, N.Y.)

In the screening of compounds for possible chemotherapeutic effects against transmitted mouse leukemia, 4-amino-N^{10}-methyl-pteroyl glutamic acid and 2,6-diaminopurine have been found to be effective in prolonging the survival time of mice with the Ak 4 strain of leukemia. Since untreated mice injected with this strain of leukemia show a marked increase in total leukocyte count and in the relative percentage of atypical prolymphocytes, it was felt worthwhile to investigate the effect of these compounds on the blood picture of normal and leukemic mice at therapeutic doses.

4-Amino-N^{10}-methyl-pteroyl glutamic acid caused slight leukopenia in normal mice but there was no alteration of the differential count. 2,6-Diaminopurine, at these doses, had no significant effect on the total leukocyte count or differential of normal mice. The average leukocyte counts of leukemic mice receiving these drugs remained significantly lower than those of the leukemic controls after the ninth day of the experiment, and the differential counts done on the eleventh to the thirteenth day showed a suppression of the atypical prolymphocytes allowing a relative increase in the number of both mature lymphocytes and neutrophils. This suppression of the leukemic process with 4-amino-N^{10}-methyl-pteroyl glutamic acid was also reflected in the microscopic appearance of section of liver, spleen, kidney, and bone marrow.

* This investigation was supported (in part) by a research grant from The National Cancer Institute of The National Institute of Health, U.S. Public Health Service, and (in part) by a research grant from The American Cancer Society.

† Fellow of The American Cancer Society, recommended by the Committee on Growth of The National Research Council.

THE RESPONSE OF ACUTE LYMPHOID LEUKEMIAS IN MICE TO 4-AMINOPTEROYL-GLUTAMIC ACID (AMINOPTERIN). L. W. Law and Thelma B. Dunn. (National Cancer Institute, Bethesda, Md.)

The effect of aminopterin has been studied on 4 transplantable lymphomas in the mouse. A detailed study of one of these, acute lymphoid leukemia, L1210, in the dba strain is presented. A statistically significant increase in survival time (approximately 40 per cent over controls), inhibition in the growth of localized leukemic mass, inhibition of infiltration into hemopoietic organs and maintenance of a normal blood picture, were found at a non-toxic optimal dosage level of 0.15 mg/kg. body weight.

The optimal dosage required for a similar response of the same leukemic cells grown in a different genetic background, DBF_1 mice was found to be 0.20 mg/kg. Aminopterin is effective in adrenalectomized mice at a somewhat reduced dosage. Synthetic folic acid, Diopterin and Teropterin did not effect the course of this leukemia in dba mice. Partial reversals of inhibition have been obtained with Teropterin at a ratio of 1:400.

Despite profound inhibition in growth and infiltration of leukemic cells, no histological differences in treated and normal leukemic cells have been noted and no toxic effects of the drug have been found in various tissues studied at the optimal dosage used. A reduction in reticulocytes to approximately $\frac{1}{4}$ the normal mouse and to $\frac{1}{10}$ the leukemic controls was noted.

TREATMENT OF ACUTE LEUKEMIA WITH A-METHOPTERIN (4-AMINO-N^{10}-METHYL-PTEROYL GLUTAMIC ACID). Leo M. Meyer, (and by invitation) Franklin R. Miller, Manuel J. Rowen, George Bock, and Julius Rutzky. (Department of Therapeutics, New York University College of Medicine, New York, N.Y. and the Department of Medicine, Jefferson Medical College, Philadelphia, Pa.)

Three children and 9 adults with acute leukemia were treated with a-methopterin (4-amino-N^{10}-methylpteroyl glutamic acid). One child showed clinical improvement. Enlarged nodes and spleen were reduced in size. The leukocytes fell from 100,000 to 10,000/cu.mm. but blast cells persisted. A second child developed severe leukopenia with hypoplastic marrow. In a third child no change in the blood picture occurred and death followed massive hematuria, rectal bleeding, and oozing of blood from gums and lips. In the adult group one patient showed clinical improvement with reduction of total leukocytes from 100,000 to 19,000/cu.mm. but blast cells remained. In another adult there was clinical improvement on two occasions, with elimination of bone pain, subsidence of temperature, reduction of total leukocytes, and increase of neutrophiles. The bone marrow remained blastic. He developed exfoliative dermatitis of the hands and feet, bleeding from gums and lips, and ulceration of tongue and pharynx while under treatment. These cleared up after the drug was discontinued. Four patients showed no hematologic or clinical improvement but demonstrated toxic effects of the drug as manifested by dysphagia, nausea, vomiting, bowel hemorrhage and buccal mucosa ulcerations. Two cases developed severe leukopenia (200 W.B.C./cu.mm.) with severe hypoplastic marrow. Another patient developed a less severe leukopenia but the marrow remained hyperplastic and blastic. All 3 cases showed oral lesions and gastro-intestinal bleeding. In 2 of the 9 adults a deep blue hemorrhagic infiltration of the skin was observed. Anemia persisted in all of the patients observed.

THE INFLUENCE OF VARIOUS DIETARY FACTORS ON THE INDUCTION OF EPITHELIAL TUMORS IN MICE.

H. P. Rusch, R. K. Boutwell, and M. K. Brush (by invitation). (McArdle Memorial Laboratory, Medical School, University of Wisconsin, Madison 6, Wis.)

The influence of specific vitamins and of less clearly defined dietary factors on the formation of cancer has been the subject of a considerable number of reports. Many of these investigations were made before the influence of caloric restriction on tumor formation was known and before much information on the exact dietary requirements of the mouse was available. The present investigation is a report of the influence of various vitamins and other dietary factors on the induction by benzpyrene of epithelial tumors in mice. The caloric intake was carefully controlled.

The mice were divided into groups of 48 and the following diets were fed: (a) all vitamins high, (b) all vitamins low, (c) thiamine-riboflavin low, (d) pyridoxine low, (e) niacin-pantothenic acid low, (f) thiamine-riboflavin-pyridoxine-niacin-pantothenic acid low, (g) pteropterin substituted for folic acid, (h) highly purified diet, (i) rice diet, and (j) whole-wheat-milk diet. The vitamin level varied from minimal requirements to over one hundred times the required level in the high vitamin group. The final incidence of tumors was essentially the same in all groups except in group b, where the incidence was decreased by about 33 per cent. The condition of the mice on all the groups remained good except for a few in group c which showed a thiamine deficiency and group b where signs of pyridoxine deficiency were noted in several mice. Such deficiencies were cured by the parenteral administration of the appropriate vitamin. The importance of controlling the caloric intake in such experiments is stressed.

ON THE STIMULATING EFFECT OF DIETARY FAT ON CARCINOGENESIS.

R. K. Boutwell, H. P. Rusch, and Miriam K. Brush (by invitation). (McArdle Memorial Laboratory, Medical School, University of Wisconsin, Madison 6, Wis.)

It is recognized that increasing the fat content of the diet tends to increase the rate of tumor formation. This phenomenon has not been adequately explained. At a fat level of 2 per cent, the incidence of epithelial tumors induced by benzpyrene in mice fed 6, 8, 10, and 12.1 calories per day was 19.5, 49, 70, and 82 per cent respectively. In the same experiment, mice fed a 28 per cent fat diet at the 6 and 10 calorie level had a tumor incidence of 30.5 and 76 per cent respectively. Forbes and coworkers have shown that there is a decreasing energy expense of utilization of the isocaloric intake of diets in the order of their increasing fat content. Using their data, it was calculated that the energy expense of utilization of the diet containing 2 per cent fat was about 3.1 calories, while the dynamic effect of 10 calories of the diet containing 28 per cent fat was about 1.6 calories. Thus the net energy value of the 28 per cent fat diet was greater by about 1.5 calories. By reference to a curve relating the tumor incidence to the caloric intake of a 2 per cent fat diet, it was seen that the degree of tumor stimulation due to the 28 per cent fat diet was of the same order of magnitude as that which resulted from an increase of 1.5 calories in the 2 per cent fat diet. It appears that the ability of fat to increase the net energy value of a diet is sufficient to explain the fat effect.

DEPENDENCE OF FORMATION OF SPONTANEOUS MAMMARY CARCINOMA IN MICE ON THE PROPORTION OF DIETARY FAT.

Albert Tannenbaum and Herbert Silverstone (by invitation). (Department of Cancer Research, Medical Research Institute, Michael Reese Hospital, Chicago 16, Ill.)

The formation of some types of tumors is accelerated by a "high" proportion of fat in the diet. It seemed worth while to examine the quantitative relationship between tumor formation and the degree of fat enrichment of the diet. For these studies the spontaneous mammary carcinoma was utilized. In one experiment employing 4 groups of 50 strain C3H mice, the proportions of dietary fat were 2, 6, 12, and 26 per cent of rations composed principally of Purina fox chow meal, skimmed milk powder, conrnstarch, and Kremax (partially hydrogenated cottonseed-soybean oil). In another experiment employing 5 groups of 60 strain dba mice, the proportions of dietary fat were 2, 4, 8, 16, and 24 per cent of rations composed principally of casein, Kremax, cornstarch, salts, and B-vitamins. In both studies the pro-

portion of fat was increased by substituting Kremax for an equicaloric amount of cornstarch; protein, salts, and vitamins remained constant in amount. The diets were fed equicalorically at slightly below *ad libitum* levels. The experiments were continued until the mice were 2 years of age.

The results of the two investigations were in excellent agreement. It was found that the rate of tumor formation (as measured both by incidences and average times of appearance of the tumors) increased with increasing proportion of dietary fat. The effect was related specifically to the proportion of dietary fat and was not caused by different caloric intakes or different body weights of the mice.

THE EFFECT OF DIETARY FAT AND CARBOHYDRATE ON DIETHYLSTILBESTROL INDUCED MAMMARY CANCER IN THE RAT.* W. F. Dunning, W. R. Curtis, and M. E. Maun (by invitation). (Department of Pathology, Wayne University College of Medicine; Detroit Institute of Cancer Research; and St. Mary's Hospital, Detroit 1, Mich.)

The effects of dietary fat were assayed under conditions of controlled caloric intake by placing 84 A×C Line 9935 female rats with diethylstilbestrol pellets implanted in their scapular regions on isocaloric synthetic rations of varying fat and carbohydrate content. Diets adequate in protein, minerals and vitamins, varying in fat content from 6.5 to 46.0 per cent with sufficient dextrin to equalize the caloric content, were fed *ad libitum* and restricted to rats in individual cages. The caloric consumption varied from 40 calories daily for rats on the *ad libitum* high fat diet to 34 calories for those on the *ad libitum* low fat diet and their paired mates on the high fat diet, and was restricted to 25 calories in isocaloric portions of high fat, modified low fat, and low fat diets.

Of the 67 rats which survived for at least 180 days, 53 or 79 per cent developed 236 gross and 337 microscopic adenocarcinomas of the mammary gland. Restricting the caloric intake by 26 to 38 per cent of the *ad libitum* consumption did not decrease the percentage of rats which eventually developed mammary cancer, but increased the latent period from approximately 300 to 400 days.

More tumors were observed in a shorter average latent period in rats on a high fat diet than in their paired mates. Increased consumption of the high fat diet, however, lessened rather than enhanced these differences and the only consistent effect appeared to be an accelerated growth potential in the preformed cancer cells.

RELATION OF DIET TO THE DEVELOPMENT OF MAMMARY TUMORS INDUCED BY FEEDING 2-ACETYLAMINOFLUORENE. R. W. Engel and D. H. Copeland (by invitation). (Laboratory of Animal Nutrition, Alabama Polytechnic Institute, Auburn, Ala.)

* Supported in part by a grant-in-aid from the U.S. Public Health Service.

Mammary tumors are consistently produced in female rats of the AES strain when subsisted on semisynthetic diets containing 0.03 per cent 2-acetylaminofluorene (see Engel and Copeland, these meetings, 1948; and Science, **108**:336–37, 1948).

Several diet modifications were employed to determine the relation of nutrition to the development of this type of tumor. Two basal diets were employed, differing in protein-content and protein-source. Basal diet C-1 contained approximately 11 per cent protein (9 per cent of casein and 20 per cent of degerminated corn grits) and basal diet C-20 contained approximately 20 per cent protein (6 per cent of casein and 30 per cent of alcohol-extracted peanut meal). Increasing the casein content of basal diet C-1 to 27 per cent resulted in more rapid body weight gains and an increase in consumption of the carcinogen. This, however, did not influence tumor incidence or time required for tumors to develop.

The addition of a detergent to basal diet C-20 at a level of 0.5 per cent appeared to enhance the action of this carcinogen; tumors appeared earlier, grew more rapidly and killed the host earlier when this diet modification was made. The addition of teropterin to basal diet C-1 (50 mg/kilo) likewise caused tumors to appear somewhat earlier. Varying the riboflavin content of basal diet C-1 (from 1 to 100 mg/kilo) failed to influence tumor incidence or induction time.

THE OCCURRENCE OF NEOPLASMS IN CHICKENS AS A RESULT OF PROLONGED CHOLINE DEFICIENCY. A. E. Schaefer (by invitation), D. H. Copeland (by invitation), and W. D. Salmon (by invitation) (Introduced by R. W. Engel). (Laboratory of Animal Nutrition, Alabama Polytechnic Institute, Auburn, Ala.)

Cancer was observed in 12 of 23 chickens that died while receiving diets low in choline. No tumors were found in seven control chickens receiving the same diets supplemented with 0.3 per cent choline chloride for comparable periods of time. This extends to another species, the previous results on the production of neoplasms in rats. (Am. J. Path., **22**:1059, 1946, Annal New York Acad. Sci., **49**:49, 1947.) Two diets, similar to those in the rat studies except for the vitamin and mineral modifications necessary for chickens, were used. At the beginning of the experiment the diets were supplemented with 0.1 to 0.3 per cent choline chloride which was reduced to 0.05 per cent or zero after 13 to 23 weeks. Neoplasms were observed after the birds received the low choline diets for 14 to 44 weeks or a total of 33 to 60 weeks on experiment. A total of 16 tumors were identified; three of these were classified as metastases. Adenocarcinoma of the liver was observed in 3 chickens and in two of these there were metastases to the intestine. One chicken had a hemangio-endothelioma of the liver with a metastasis to the ovary. Cholangioma of the liver was found in one bird. One fibromyxoma and two embryonal nephromas were observed in the kidneys of 3 chickens. Five subcutaneous tumors were found. Four of these were fibrosarcomas and one was a benign fibroma.

PHOSPHAMIDASE IN NEOPLASMS. George Gomori. (Dept. of Medicine, University of Chicago, Chicago 37, Ill.)

Results obtained with the new histochemical reaction for phosphamidase (Proc. Soc. Exper. Biol. and Med., In press) are presented. This enzyme occurs in small to moderate amounts in all tissues but in large amounts only in the gray matter of the central nervous system and in malignant epithelial tumors. In over 60 carcinomas of all kinds intense differential staining of the malignant change, setting it off against its environment, was obtained. The intensity of the reaction generally parallels morphologic criteria of malignancy, although sometimes fairly intense reactions are obtained in rectal polyps, not malignant morphologically. In sarcomas the results are variable; some of them stain just like carcinomas, while others show a patchy reaction or none at all.

THE EFFECT OF CERTAIN STEROLS ON SUCCINOXIDASE, AND GLYCOLYSIS OF TUMOR SUBCELLULAR (PARTICULATE) FRACTIONS. Kent Wight (by invitation), and Dean Burk. (National Cancer Institute, Bethesda, Md.)

It has been found that certain hormones act competitively on the succinoxidase system of the mitochondrial elements of the S91 and Harding-Passey melanomas grown in dba and C mice respectively. The mitochondrial elements (microscopically visible cytoplasmic particulates, mostly melanized; see Woods, Du Buy, Burk, Hesselbach, and Lackey, in J. Nat. Cancer Inst., Feb. 1949) were obtained by centrifugation at 10° C. of saline, glass wool-filtered tumor homogenates at 10,000*g* for 30 minutes after preliminary centrifugation at 25*g* for 10 minutes to eliminate nuclei and heavy phagocytized cell material. The tumors used for studies of glycolysis were homogenized in isotonic sucrose. Oxygen consumption was measured manometrically after addition of 0.05M phosphate (pH7), 0.00001M cytochrome c, and 0.02M succinate. Glycolysis was measured by CO_2 liberation from a system consisting of a nutrient solution (made according to Le Page, J. Biol. Chem., **176:** 1021–27, 1948) and the sterol.

Diethylstilbestrol, at a concentration of ~ 135/ml. (=0.0005M), inhibited particulate oxygen consumption 60 to 75 per cent. This inhibition was markedly reduced or entirely eliminated by the simultaneous addition of ~125/ml. (=0.0002M) progesterone or testosterone. Progesterone alone had no effect on oxygen consumption. S91 and Harding-Passey tumor particulates behaved similarly except for differences in the oxygen uptake. Diethylstilbestrol also markedly inhibited or eliminated oxygen consumption by mitochondrial elements from tumors such as the C3HBA breast carcinoma and sarcoma 37 grown in C3H and C mice respectively.

Anaerobic glycolysis was stimulated 50 per cent in the case of the C3HBA breast carcinoma by diethylstilbestrol.

β-GLUCURONIDASE ACTIVITY AND LACTIC ACID CONTENT OF BODY FLUIDS OF PATIENTS WITH AND WITHOUT CANCER.* William H. Fishman, Richard L. Markus, and Paul H. Pfeiffer (by invitation), and F. Homburger. (Laboratories of the Cancer Research and Cancer Control Unit of the Department of Surgery and the Departments of Biochemistry and Medicine, Tufts College Medical School, Boston, and the Department of Clinical Research, Jewish Memorial Hospital, Roxbury, Mass.)

Ordinarily those constituents of neoplastic exudates which derive from the metabolism of tumors are not in equilibrium with the same substances in the blood, because of their more rapid genesis in the tumor and entry into the exudate, and because of the slow rate of return of such components into the circulation. Therefore, it is reasonable to expect that the composition of pleural and ascitic fluids occurring in the presence of malignancies would reflect the biochemical changes peculiar to the tumors. Substances in such fluids which occur in higher or lower concentration than in the blood thus are of metabolic interest in the study of malignancy.

As a part of a systematic investigation on the significance of the chemical composition of fluids and secretions in human cancer, the β-glucuronidase activity and the lactic acid content of a number of body fluids have been studied. Measurements of β-glucuronidase activity were chosen because of the previous observations of one of us (W. H. F.), which indicated the presence in the majority of cases studied of higher levels of β-glucuronidase in cancer tissue than in the tissue of origin. Lactic acid was determined because of the well-established ability of tumor tissue to produce lactic acid from glucose by anaerobic glycolysis. Wherever possible the sediment of the fluid prepared by centrifugation was stained according to the Papanicolaou technique and studied microscopically. Pathological confirmation was obtained, in addition, by autopsy in a high percentage of cases.

In a significant number of fluids from patients with carcinomatosis of the chest or abdominal cavities, both the glucuronidase and the lactic acid values were considerably elevated. In some, either one or the other showed abnormal values, and in others neither was abnormal. Determination of lactic acid by quantitative chemical methods was found essential, the usual clinical qualitative methods being entirely unreliable. Chemical and enzymological observations have been correlated with the clinical status of the patient, especially with regard to previous therapy The considerations which led to this work on fluids contained in closed body cavities apply in part also to body secretions and the same studies are being applied at present in this laboratory to gastric juice and vaginal secretions. The possible diagnostic value of such determinations cannot of course be ascertained until sufficient comparative studies have been made on secretions, transudates and exudates in patients without cancer.

* This work was aided by an institutional grant of the American Cancer Society and by a research grant from the National Cancer Institute of the National Institute of Health, U.S. Public Health Service.

AN ANALYSIS OF THE β-GLUCURONIDASE CONTENT OF CIRCULATING WHITE BLOOD CELLS OBTAINED FROM HUMAN LYMPHOBLASTOMATOUS AND CONTROL WHOLE BLOOD SAMPLES. A. JOHN ANLYAN and JESS F. GAMBLE (by invitation), and HERMAN A. HOSTER. (Ohio State University College of Medicine, Columbus, Ohio)

The work of Fishman and Anlyan has demonstrated a greater than normal content of the enzyme β-glucoronidase in human neoplastic tissues. It has further been shown that the enzyme β-glucuronidase is present for the most part in the buffy coat of human blood. The purpose of the present study is a quantitative estimation of the β-glucuronidase content of the buffy coat of human Hodgkin's disease and leukemic bloods. As a control measure, a number of determinations using the blood of normal human subjects and patients with various malignant conditions other than lymphomata were made.

A new method for separation of the buffy coat from the other elements of human whole blood has been devised by the authors. The β-glucuronidase activity per gram of buffy coat in patients with Hodgkin's disease, lymphatic leukemia, monocytic leukemia, and myelogenous leukemia is determined with repeat determinations during and after therapy. The results at the present time indicate that the buffy coat β-glucuronidase activity of patients with the leukemias and Hodgkin's disease varies significantly from the activity observed in the normal control group.

ACID PHOSPHATASE ACTIVITY OF THE GASTRIC CONTENTS OF PATIENTS WITH CARCINOMA OF THE STOMACH. CHARLES E. DUNLAP and GEORGE W. CHANGUS (by invitation). (Department of Pathology, School of Medicine, Tulane University and Charity Hospital, New Orleans, La.)

In patients with carcinoma of the stomach histochemical studies by Gomori have shown high acid phosphatase activity in the neoplasm and also in the surrounding gastric mucosa. Chemical determinations for acid phosphatase were done by the method of Gutman and Gutman on gastric contents, aspirated from a series of fasting patients with and without carcinoma of the stomach. It was found that aspirates having an initial pH of less than 3.5 seldom contained significant amounts of acid phosphatase and that the enzyme, when present in other samples, could be irreversibly inactivated, *in vitro*, by acidification to pH 3.5 or less. At a pH greater than 3.5 the enzyme was fairly well preserved for 48 hours at 4° C. but rapid deterioration occurred at room temperature. Thus gastric aspirates containing "free acid" (pH less than 3.5) as well as those that had stood for more than 2 hours without refrigeration were considered unsuitable for acid phosphatase determinations. In a great majority of the patients with carcinoma of the stomach no "free acid" was present and the aspirates were found to contain more than 10 units of acidphosphatase per hundred cc. Most samples from patients without gastric carcinoma contained less than 10 units. The study, to date, has covered only a limited number and variety of gastric lesions and includes no cases of early carcinoma.

PURINE METABOLISM IN TETRAHYMENA AND ITS RELATION TO NEOPLASTIC TISSUE. G. W. KIDDER, VIRGINIA C. DEWEY, and R. E. PARKS, JR., GILBERT L. WOODSIDE (Introduced by J. C. Aub). (Biological Laboratory, Amherst College, and Zoological Laboratory, University of Massachusetts, Amherst, Mass.)

The ciliated protozoan, *Tetrahymena geleii*, has been shown to have a requirement for the purine, guanine, or its riboside. A series of purine analogues has been tested as inhibitors of purine metabolism. Of 16 analogues tested, 6 proved to have an inhibitory effect. Of these six, 4 were xanthine analogues. The inhibition indices were: caffeine, 100; theobromine, 150: theophylline, 225; and paraxanthine, 300. None of these inhibitions was completely reversed by guanine. The remaining compounds, 5-amino-7-hydroxy-1-v-triazolo-(d)-pyrimidine and 5-7-diamino-1-v-triazolo-(d)-pyrimidine had indices of 0.075 and 85 respectively.

Recent tests on another series of twelve analogues showed that 2,6,8 triamino purine had an inhibition index of about 15. On the other hand, 2,4 diamino-5-formyl-amino-6 hydroxypyrimidine spared guanylic acid. This demonstrates the ability of the organism to synthesize guanine and/or adenine by completing the imidazole ring. It appears that water is split out from the adjacent aldehyde and amino groups in the 5 and 4 positions respectively. Acting on the assumption that neoplastic tissue differs from normal animal cells in its guanine requirement, the most effective compounds of this series were tested. Positive results of these tests which have been obtained will be reported.

THE APPLICATION OF DIFFERENTIAL CENTRIFUGATION AND ELECTRON MICROSCOPY TO THE SEGREGATION AND VISUAL STUDY OF HUMAN LYMPH NODE CELL MACROMOLECULAR PARTICLES IN THE SIZE RANGE OF 20 TO 300 mμ. MIRIAM S. HOSTER, BETTE J. MCBEE, and HARRY A. ROLNICK (by invitation), and HERMAN A. HOSTER. (The Ohio State University College of Medicine, Columbus, Ohio)

Since no information concerning the segregation and visual study of human lymph node cell macromolecular components in the size range of 20 to 300 mμ was available in the literature, the present study was undertaken to develop a satisfactory technique for this purpose. Differential centrifugation was used to separate these lymph node cell components obtained from patients with Hodgkin's disease and control diseases of diverse etiology. After each step in the separation procedure, a study of these bodies was made in the electron microscope.

The research tools utilized in this study were the ultra-centrifuge, the dark field microscope and the elec-

tron microscope. Chemical and spectrophotometric studies were included as a supplementary guide in component separation. A number of separation techniques will be presented with the significant factors involved in each. These considerations include the type of diluent used, the hydrogen ion concentration, the duration of the extraction period at 4° C. and the methods of differential centrifugation. Calcium chloride and osmium vapor staining of formvar coated electron microscope screens whose surfaces are covered with a fine film of the sample to be studied will be discussed. A description of the preliminary observations recorded to date is included.

EFFECT OF INOCULATION OF THE VIRUSES OF INFLUENZA, HERPES, AND RUSSIAN FAR EAST ENCEPHALITIS ON THE GROWTH OF TRANSPLANTABLE TUMORS IN MICE. Alice E. Moore (Introduced by C. P. Rhoads). (Division of Experimental Chemotherapy, Sloan-Kettering Institute, New York, N.Y.)

Three viruses of widely different characteristics were studied for their ability to grow in transplantable mouse tumors and to determine the effect of such a parasitization in the viability of the tumor.

It was found that both the viruses of influenza and herpes could grow for a limited period when inoculated directly into sarcoma 180. Their presence had no effect on the growth or transplantability of the tumor. There was no evidence that these viruses had any special affinity for either sarcoma 180 or the mouse adenocarcinoma EO 771.

In contrast, the virus of Russian Far East Encephalitis not only rapidly parasitized sarcoma 180 but showed a definite preference for the neoplasm. In the process of viral infection tumor growth was definitely inhibited. Complete destruction of the tumor, which could be demonstrated by bioassay into virus immune mice and by cytological study was always associated with systemic infection and death of the animal.

ISOLATION OF THE MOUSE MAMMARY CARCINOMA VIRUS. Samuel Graff, Wendell M. Stanley, Dan H. Moore, and Henry T. Randall (by invitation), and Cushman D. Haagensen. (College of Physicians and Surgeons, Columbia University, New York, and Rockefeller Institute For Medical Research, Princeton, N.J.)

Characteristic sub-microscopic particles have been isolated from milk of high cancer strain mice. This material could not be detected in the milk of a cancer free strain. Minute amounts of this material produce carcinoma in otherwise cancer-free mice. Similar particles were isolated from the milk of low cancer strain mice after they were foster-nursed on high cancer strain mothers.

AN IMPROVED METHOD FOR THE STUDY OF CERTAIN METABOLITES OF THE CARCINOGENIC AZO DYES. Nelson F. Young. (Samis Grotto Cancer Research Laboratory, Medical College of Virginia, Richmond, Va.)

One phase of investigation of the problem of butter-yellow carcinogenesis in rats has been the demonstration of certain metabolites of the dye *in vivo* and *in vitro*. The methods by which such metabolites have been isolated and quantitated are somewhat tedious and require the use of special reagents and equipment. By an adaptation of the technique of paper chromatography, such separations may be made conveniently and quickly. The method requires no special equipment or reagents and the sensitivity compares favorably with existing methods. Since the separations are followed visually, the modifications necessary for the isolation of new compounds or work on dyes other than butter-yellow are rapidly and readily made. A comparison of the *in vitro* metabolites of several carcinogenic and closely related non-carcinogenic dyes will serve to demonstrate the usefulness and limitations of the method.

DISTRIBUTION OF PHOSPHORUS-CONTAINING COMPOUNDS IN MAMMARY GLANDS AND MAMMARY TUMORS OF MICE BY RADIOBIOLOGICAL METHODS. S. Albert, and Ralph M. Johnson, and Patricia J. Keller (by invitation). (Richard Cohn Radiobiology Laboratory of the Detroit Institute of Cancer Research, Detroit 1, Mich.)

An attempt has been made to determine some of the changes in the metabolism of phosphorus-containing compounds accompanying mammary cancer development. Radioactive phosphorus was injected into tumor-bearing high cancer strain and into non-tumor bearing low cancer strain female mice; 17 hours later they were sacrificed. Phosphorus-containing compounds of mammary glands and tumors were separated by a method modified from those of Schneider and Schmidt, and Thannhauser.

The phosphorus in tumors was evenly distributed between the acid-soluble, desoxyribonucleic and ribonucleic acid fractions, and was lowest in the phospholipid. In mammary glands the phosphorus was highest in the acid-soluble fraction, lower in the phospholipid, and lowest in the ribonucleic and desoxyribonucleic acids.

The radioactivity was found to have the same general distribution in mammary glands as in tumors. There was a definite shift, however, of radioactivity from the acid-soluble to the acid-insoluble fraction in tumors. This was evident in the desoxyribonucleic and ribonucleic acids but not in the phospholipid. At this interval after injection, the radio phosphorus per microgram of phosphorus was about twice as high in the tumor desoxyribonucleic acid, ribonucleic acid, and phospholipid fractions as in the corresponding fractions of non-cancerous mammary glands. The uninvolved mammary glands of tumor-bearing cancer susceptible females contained more phosphorus on a wet weight basis than did those of cancer resistant animals. This difference disappeared, however, when expressed on the basis of total nitrogen, probably indicating more glandular tissue per unit weight in the mammary glands of cancer susceptible animals.

AGE AND STRAIN DIFFERENCES IN PHOSPHORUS METABOLISM IN VARIOUS ENDOCRINE ORGANS OF MICE. Ralph M. Johnson (by invitation), and S. Albert. (Richard Cohn Radiobiology Laboratory of the Detroit Institute of Cancer Research, Detroit 1, Mich.)

The metabolic activity of endocrine glands in high and low cancer strains of mice may shed light on the relationship of endocrine activity to the process of carcinogenesis. The important role of phosphorus in cellular metabolism suggests that the uptake of this element (as radioactive phosphorus) can be used as a measure of the functional activity of such endocrine glands.

Mice of the C57 and dba strains were injected with radioactive phosphorus (P^{32}) as inorganic phosphate, and killed at intervals up to 48 hours following the injections. Pituitaries, thyroids, adrenals, testes, and blood were removed, digested, and aliquots taken for both radioactivity assay and colorimetric determination of P^{31}.

Based upon accumulation of radioactive phosphorus, the testes of both strains were the least active followed by the pituitaries, thyroids, and adrenals, increasing in that order. Age variations did not alter this relationship. The adrenals of the immature dba mice were more active than those of C57 mice of a comparable age. The reverse was true, however, in the mature animals. The testes of the immature animals of both strains accumulated phosphorus to the same extent. In the older animals, the phosphorus uptake by testes in C57 mice exceeded that found in the mice of the dba strain. Within either strain, the adrenals and testes of the immature animals accumulated more phosphorus than the same organs of the mature animals. The uptake of radiophosphorus by pituitaries and thyroids appeared to be independent of both the age and strain of the animals.

LOCALIZATION OF RADIOACTIVE COMPOUNDS IN TUMORS. Wm. G. Myers. (College of Medicine, The Ohio State University, Columbus, Ohio)

Several hundred intermediate compounds are being collected and purified, or synthesized, to which are coupled radioactive sulfanilic acid labelled with S^{-35} and iodinated derivatives and isomers of it labelled with I^{-131}, by azo linkages. Most of the products are radioactive acidic or basic dyes of widely varied structures which contain several functional groups. Purification of these is readily carried out by chromatographic adsorption methods.

A rapid scanning procedure has been evolved for the comparison of the relative concentrations of radioactivity in the liver, spleen, kidney, skeletal muscle, blood, and the transplanted tumors, C3HBA adenocarcinoma, 15091a spindle cell mammary carcinoma, and sarcoma 37 in C3H, ABC, and CFW mice respectively, at 15 minutes, 1 hour, 4 hours, and 25 hours after the intravenous injection of approximately 1 mg. of each of the radioactive compounds.

Two of the forty S^{-35} labelled compounds tested to date have shown concentrations of radioactivity in the carcinomas up to double those in the blood and five times the concentrations in the muscles at one or more of the intervals after injection. More extensive tests on these compounds and derivatives and isomers of them are in progress.

THE DISTRIBUTION OF RADIOACTIVITY AND THE METABOLIC DEGRADATION IN THE MOUSE OF 20-METHYLCHOLANTHRENE-11-C^{14}. William G. Dauben and Dorothea Mabee (Introduced by Hardin B. Jones). (Department of Chemistry and Radiation Laboratory, University of California, Berkeley, Cal.)

These experiments were undertaken to study the distribution and metabolism of methylcholanthrene and to compare these findings with the dibenzanthracene results of Heidelberger and Jones.

Strain A male mice were injected with methylcholanthrene-C^{14}. Radioactivity was found in all tumors and in 24 per cent of the tumors more than 5 per cent of the injected dosage was present. The amount of methylcholanthrene injected had no effect upon the percent of activity found in the tumor. The tumors were then subjected to chemical analysis. The amount of methylcholanthrene which was found in the tumor was of the same order of magnitude as that reported in the dibenzanthracene study. It was also found that the distribution of the degradation product of methylcholanthrene followed the same pattern as dibenzanthracene. Since the metabolism of methylcholanthrene and dibenzanthracene is similar and whereas the carcinogenic index of methylcholanthrene is three times that of dibenzanthracene, it can be concluded that methylcholanthrene is three times more active and metabolizes three times more rapidly.

Site of injection, liver, fat, and muscle were removed at intervals for rate studies. At the end of 7 weeks, 50 per cent of the injected dose had disappeared from the injection site. Activity in liver, fat, and muscle was absent or negligible. The rate of elimination in urine and feces was determined for the first week following injection, and totally 1 to 3 per cent of the injected dose was eliminated in the urine and approximately 10 per cent in the feces.

USE OF STARCH COLUMN CHROMATOGRAPHY IN STUDY OF AMINO ACID COMPOSITION AND DISTRIBUTION OF RADIOACTIVITY IN PROTEINS OF NORMAL RAT LIVER AND HEPATOMA. Paul C. Zamecnik, and Ivan D. Frantz, Jr., and Mary L. Stephenson (by invitation). (From the Medical Laboratories of the Collis P. Huntington Memorial Hospital of Harvard University at the Massachusetts General Hospital, Boston, Mass.)

Animal tissue is composed of many proteins of varying amino acid composition. In the synthesis of these proteins and the direction of the sequential arrangement of the peptide bound amino acids it is likely that a number of highly specific enzymes participate. The possibility thus suggests itself that a change might occur in the proteosynthetic enzymes of malignant tissue which

would be reflected in an altered amino acid composition of the proteins synthesized. By means of starch column chromatography, and with the help of Drs. Stanford Moore and William Stein, comparisons have been made of the amino acid composition of trichloroacetic acid precipitated proteins obtained from primary rat hepatomas and from normal rat livers. Chromatograms from the normal and malignant tissues have been found to be in most respects superimposable. A small but significant increase in the leucine-isoleucine peak has, however, been found in the hepatoma.

In a series of *in vitro* experiments, C^{14}-carboxyl-labeled alanine, C^{14}-carboxyl-labeled glycine, and $NaHC^{14}O_3$ have been added to tissue slices of normal liver and of hepatoma. Starch column chromatograms have been made of hydrolysates of the slice proteins at the end of the experiments. In the $NaHC^{14}O_3$ experiments, radioactivity has been found predominantly in aspartic and glutamic acids, and in arginine. Smaller amounts of radioactivity have also been found in glycine and serine, indicating carbon dioxide fixation in these amino acids in a manner as yet unexplained and heretofore unknown. Preliminary experiments suggest greater fixation of carbon dioxide in glycine in the hepatoma than in the normal liver slice. The fixation of carbon dioxide in the guanidine group of arginine is decreased in the hepatoma.

AMINO ACIDS IN HYDROLYSATES OF THE MITOCHONDRIAL FRACTION IN NORMAL AND NEOPLASTIC TISSUES AS STUDIED BY PAPER CHROMATOGRAPHY. Eugene Roberts and Chao-T'E Li (by invitation). (Department of Anatomy, Division of Cancer Research, Washington University Medical School, St. Louis, Mo.)

Mitochondria were isolated from normal mouse liver, pancreas, and kidney and from mouse mammary and squamous cell carcinomata and hepatoma by differential centrifugation according to the method of Hogeboom *et al.* The isolated particles were the same size and shape as the structures identified as mitochondria in smears made from homogenates and in free cells found in the sediment from the first low-speed centrifugation, and they possessed the same staining characteristics with Janus green B before fixation and with aniline-acid fuchsin after fixation with osmic acid.

Aspartic acid, glutamic acid, glycine, alanine, valine, the leucines, serine, and proline were the amino acids present in greatest quantities after hydrolysis of the mitochondria for 24 hours in 6N HCl in a sealed tube at 109° C. Threonine, tyrosine, phenylalanine, cystine, histidine, and arginine were present in considerably smaller quantities. The results indicate a large excess of the dicarboxylic amino acids over the basic amino acids. The patterns found for the different tissues will be shown and the similarities and differences in the normal and malignant tissues will be discussed.

AMINO ACIDS IN CERTAIN NORMAL AND NEOPLASTIC TISSUES. H. E. Sauberlich and C. E. Blades (by invitation), and C. A. Baumann. (Department of Biochemistry, University of Wisconsin, Madison 6, Wis.)

Representative normal and tumor tissues were hydrolyzed with acid or alkali for various periods of time and the hydrolysates analyzed microbiologically for 18 amino acids. The tumors included the Flexner-Jobling carcinoma, spontaneous mammary adenocarcinoma of the rat, sarcomas of the rat induced by methylcholanthrene, and hepatomas induced by azo dyes. The normal samples included muscle and liver from normal rats, and liver from rats fed restricted amounts of food or fed a non-carcinogenic azo dye.

Each of the 18 amino acids was found in every sample analyzed. The amounts ranged from 12 per cent for glutamic acid (calculated to 16 per cent N in the moisture-free, fat-free, residue) to approximately 1 per cent for tryptophan and cystine. All other amino acids were present in intermediate amounts. For most amino acids optimal amounts were found after 3 to 8 hours of hydrolysis. In general the percentages of the individual amino acids found in the tumor samples did not differ greatly from those found in normal rat liver or rat muscle, or from the values reported by others for representative cuts of beef or pork.

However, the spontaneous mammary tumors of the rat proved to be exceptional in that they contained abnormally high amounts of glycine (17.5 per cent) and proline (10 per cent) and relatively low amounts of histidine and methionine. The discrepancy is attributed to the connective tissue present in the latter tumors.

THE EFFECT OF COBALT ON THE CORRELATION OF NUCLEIC ACID CONCENTRATION WITH RATE OF GROWTH. Hilton Levy, Elizabeth Skutch, and Arthur L. Schade (introduced by Dean Burk). (Overly Biochemical Research Foundation, Inc., New York, N.Y.)

The concentration of pentose nucleic acid (PNA) and of desoxypentose nucleic acid (DNA) has been studied as a function of the rate of growth of *Proteus vulgaris*. During early logarithmic growth of the organism in meat extract broth, the concentration of PNA whose initial value ranged from 6 to 12 per cent of the dry weight, increased two to three fold. The concentration of PNA followed closely the synthetic activity of the culture, expressed as per cent increase in dry weight per hour. The DNA concentration varied (3 to 6 per cent on dry weight basis) over the period of growth studied and showed no correlation with the growth rate.

Cells of *Proteus vulgaris*, inoculated into meat extract broth containing growth-inhibitory concentrations of cobalt, behaved like resting cells in that they did not increase in size, nor divide, nor show the increased Q_{O_2} characteristic of normal growing cells. The changes in PNA concentration, on the other hand, followed a course that was essentially identical to that of growing bacteria, *i.e.* a 2 to 3 fold increase in PNA concentration occurred at the same rate as in the control cultures. Such increase in dry weight of the culture as was found over the period of observation could be accounted for largely by the increase in the amount of PNA. The DNA

concentration of the cobalt inhibited cells varied in a manner similar to that of the control culture.

APPLICATION OF CHROMATOGAPHY TO THE SEGREGATION OF SUBCELLULAR PARTICULATES. VERNON T. RILEY (by invitation), MARIE L. HESSELBACH, M. W. WOODS, and DEAN BURK. (National Cancer Institute, Bethesda, Md.)

Melanized granules of the Cloudman S91 and Harding-Passey mouse melanomas can be reversibly adsorbed on celite columns and are thus subject to chromatographic manipulation. As a consequence, certain other constituents of the tumor homogenates can be readily separated from the granules, thereby providing a basis for non-centrifugal segregation of a substantial portion of the other tissue components. The granules so separated appear essentially as a homogeneous population in the phase contrast microscope. Granules of the Harding-Passey tumor examined in the electron microscope were also essentially free of contaminating microsomal elements and particulate debris. The mean diameter of these chromatographed granule preparations was 0.3 to 0.4 microns, with a size range of approximately 0.2 to 0.6 microns.

The particulates separated from the S91 melanoma by chromatography were studied enzymatically with respect to succinoxidase and dopa oxidase with a $QO_2(N)$ increase of approximately 6 fold and 10 fold respectively when compared to the starting extract partially purified by centrifugal clearance.

The adaptation of chromatography to subcellular particulates ranging from the chicken tumor agent in the virus range to melanized granules in the mitochondrial and bacterial range provides another method of separating and characterizing intracellular particulates.

PHYSICAL CHEMICAL STUDIES ON SERA OF MYELOMA PATIENTS.* KURT G. STERN (by invitation), DANIEL LASZLO, and JOSEPH S. KRAKAUER (by invitation). (Dept. of Chemistry, Polytechnic Institute of Brooklyn and Division of Neoplastic Diseases, Montefiore Hospital, New York, N.Y.)

The examination of the sera from 7 cases of multiple myeloma in the electrophoresis apparatus, using the Svensson optical system, revealed in 6 cases the presence of a large amount of protein component migrating with the mobility of gamma globulin. Five of the patients exhibited a hyperproteinemia, with a total protein concentration ranging from 8.5 to 14 per cent, while 2 sera showed a normal total protein content. One of the latter sera yielded a nearly normal electrophoresis diagram while the other was found to have a low albumin and a high gamma globulin concentration.

The protein fraction of lowest mobility was isolated by preparative electrophoresis from the serum of a patient in which it amounted to 70 per cent of the total protein present. The purified protein fraction was further characterized by measuring the sedimentation rate in the analytical ultracentrifuge, employing the Philpot optical system ($s_{20} = 6 \times 10^{-13}$); the rate of free diffusion in a Claesson cell, using the schlieren-scanning method of Longsworth, the relative viscosity in a capillary viscosimeter by a photographic recording method ($\eta_{sp}/c = 0.1$), as well as the ultraviolet absorption spectrum in a Beckman quartz spectrophotometer (ϵ_{max} at 278–282 mμ), were also determined.

The outstanding property of the protein studied by us appears to be its remarkable ultracentrifugal paucior monodispersity and its electrophoretic homogeneity which serve to distinguish the material from normal human gamma globulin.

* (This investigation was supported (in part) by a research grant from the American Cancer Society and the National Cancer Institute of the National Institute of Health, U.S. Public Health Service.)

CALCIUM AND POTASSIUM CONTENT OF REGENERATING TISSUES. DALE REX COMAN, and (by invitation) ROBERT P. DELONG, and I. ZEIDMAN. (Department of Pathology, University of Pennsylvania, School of Medicine, Philadelphia 4, Pa.)

Observations by others have revealed a local deficiency of calcium in cancerous tissue. In our laboratory the calcium deficiency of cancer cells has been related to a decreased adhesiveness of these cells, which in turn we have related to the invasive propensities of malignant tumors. The present preliminary study was an attempt to determine whether calcium deficiency is always associated with a state of active cell multiplication, or whether its existence in cancerous tissue is dependent upon some other features of the neoplastic state. The material used consisted of normal and regenerating rat livers. Flame photometric determinations of the calcium, sodium, and potassium content of these tissues were made. No significant differences were found in the calcium content, although regenerating tissue had a higher potassium content than the normal. This result is consistent with the hypothesis that the low calcium content of cancer tissue depends upon some other property than the rate of cell multiplication.

MINERAL METABOLISM IN METASTIC BONE CANCER.* DANIEL LASZLO. (Division of Neoplastic Diseases, Montefiore Hospital, New York, N.Y.)

Patients suffering from cancer metastases to the bones were studied under controlled metabolic conditions. Calcium, phosphorus, and nitrogen balances were determined in patients with predominantly osteolytic and osteoblastic metastases respectively. The chemical data were correlated with the clinical course and with the therapeutic measures.

Hypercalcemia is frequently associated with metastatic malignancy. Among 70 breast cancers with bone metastases, studied within one year, 10 were found to have hypercalcemia. The importance of early recognition and early treatment of this complication is illustrated and the metabolic data are presented. Osteolytic

* This investigation was supported by a research grant from the American Cancer Society.

metastases appear to be characterized by high urinary calcium concentration, high urinary calcium output and a negative calcium balance. In osteoblastic metastases secondary to prostatic malignancy a low calcium concentration in the urine, a low urinary calcium output and calcium retention were observed. The effect of androgens and estrogens on the mineral metabolism of such patients is illustrated.

THE SOURCE OF TUMOR NITROGEN IN RATS BEARING WALKER CARCINOMA 256. CHARLES D. SHERMAN, JR. (by invitation), and G. BURROUGHS MIDER. (Department of Surgery, The University of Rochester School of Medicine and Dentistry, Rochester 7, N.Y.)

Earlier experiments in our laboratory indicated that the nitrogen content of Walker carcinoma 256 exceeded the amount of dietary nitrogen stored by the host during the period of tumor growth. Therefore, most of the nitrogen in the tumor must have been obtained from the rat's tissues. The experiment to be reported was designed to show which tissues contributed nitrogen to the neoplasm.

The wet and dry weights and nitrogen content of the livers, spleens, lymph nodes, kidneys, and hearts were determined in 20 rats bearing the Walker tumor and in pair fed normal controls of the same age, weight, and sex. The remainder of each carcass ("residue") was analyzed for nitrogen content. The results indicate that most of the tumor nitrogen was contributed by the carcass "residue." The lymph nodes of the tumor-bearing rats lost 50 per cent more nitrogen than did the pair fed controls while no significant differences were found in the weights or nitrogen content of the spleen and kidneys in the two groups. The heart gave up nitrogen only in the rats with the largest tumors. The livers of the tumor-bearing rats, however, contained significantly more nitrogen than did the livers of the pair fed controls.

MELANOMA IN DOGS. R. M. MULLIGAN (Introduced by H. L. STEWART). (University of Colorado Medical Center, School of Medicine, Denver, Colo.)

Of 31 dogs with 36 melanomas (17 non-cancerous and 19 cancerous), the sex (19 male and 11 female) and age (6 to 14 years in 27) were known in 30. The breeds affected usually had deeply or moderately pigmented skin. The primary neoplasm was found on the head (8 on the eyelids and 4 in the cheeks), the thorax, and the extremities in 29 cases. The smaller tumors (less than 6 cc. in volume) were often non-cancerous and the larger (greater than 8 cc. in volume) were often cancerous. The characteristics of the neoplastic cells and of their growth pattern, the tendency to invade, and the metastasis were helpful in differentiating the cancerous from the non-cancerous melanomas.

STUDIES ON SPONTANEOUS NEOPLASMA IN FISHES. IV. GANGLIONEUROMA IN THE MARINE FISH, *HALICHOERES BIVITTATUS* (BLOCH), FROM BIMINI, B.W.I. ROSS F. NIGRELLI. (New York Aquarium, New York Zoological Society, New York, N.Y.)

Ganglioneuromatas in the Slippery Dick appear as nodules or raised wavy patches on the surface of the body. They vary in size from a few millimeters for the nodules to several centimeters for the patches. The growths are well vascularized and occasionally heavily pigmented, especially if they occur in regions that normally show a heavier concentration of melanophores (*e.g.* the lateral line). Histologically, the growth is composed of adult myelinated ganglion cells together with their processes and supported by a well developed reticulum. The hyperplasia extends from the corial region into the epithelium and over the scales. There is a tendency to infiltrate the adjacent tissues and where the growth is found on the fins, the bony rays of these appendages are invaded and replaced by the tumor cells. No metastases, however, were noted. Attempts to transplant the tumors to other areas in the same fish, and in normal fish, were without success. Seventeen hundred and eighty-eight fish were examined and 1.57 per cent were found with one or more of these growths on the body. The incidence was greater among females than either males or immature fish.

EFFECT OF VASCULAR OCCLUSION ON TRANSPLANTED TUMORS. JULIUS S. YOUNGNER and GLENN H. ALGIRE. (National Cancer Institute, Bethesda 14, Md.)

Previous work of the authors has indicated the importance of host vascular reactions, probably including local tissue anoxia, in the mechanism of action of certain tumor-damaging substances. In the present study, mechanical interference with tumor blood supply was used to simulate changes in circulatory supply resulting from injection of the tumor-damaging substances previously studied.

Microscopic observations *in vivo* were made of the reactions of normal and growing neoplastic tissues included within transparent chambers in C3H mice, prior to and following mechanical occlusion of the blood supply to the window area.

In the case of control windows without tumor transplants, vascular occlusion for periods of from 1 to 3 hours resulted in no significant visible changes in any of the tissues under observation. Vascular levels returned to normal rapidly.

When the blood supply to windows bearing sarcoma transplants was obstructed for similar periods, hemorrhage developed in the tumors within 1 hour after flow was resumed and necrosis was observed 24 hours later. Normal tissues surrounding the sarcoma were unaffected. In all cases, renewed vascularization and growth of peripheral sarcoma cells occurred. In contrast, mammary carcinomas treated in the same manner were not affected, vascular levels rapidly returned to normal without any signs of hemorrhage or necrosis.

These findings are discussed in relation to: (a) importance of host circulatory reactions in tumor damage caused by certain substances; (b) differences in response of vascular networks of sarcomas and carcinomas under the conditions of the experiments reported.

THE EFFECTS OF SEVERAL AMINOAZO DYES ON THE INTRACELLULAR COMPOSITION OF RAT LIVER. J. M. PRICE (by invitation), E. C. MILLER, J. A. MILLER, and G. M. WEBER (by invitation). (McArdle Memorial Laboratory, University of Wisconsin, Madison 6, Wis.)

Homogenates of the livers of rats fed various aminoazo dyes for four weeks were separated by differential centrifugation into nuclear, large granule, small granule, and supernatant fluid fractions. The fractions and whole homogenates were analyzed for protein, nucleic acids, riboflavin, and protein-bound aminoazo dye. The data have been compared with earlier data (Price *et al.*, J. Biol. Chem., **173**:345, 1948; Cancer Research, **9**, In press) on the same fractions from normal liver and from liver tumors induced by 4-dimethylaminoazobenzene. Dyes which are more or less carcinogenic than 4-dimethylaminoazobenzene produced greater or lesser changes, respectively, than 4-dimethyl aminoazobenzene. Thus, a carcinogenic dye (*e.g.*, 3′-methyl-4-dimethylaminoazobenzene) tended to make the liver more like tumor tissue while the non-carcinogenic 4-aminoazobenzene did not alter the composition of the liver. Increased contents of desoxypentosenucleic acid and protein in the nuclear fraction, decreased contents of protein, riboflavin, and pentosenucleic acid in the large granules, a decreased content of pentosenucleic acid in the small granules, and an increased pentosenucleic acid content in the supernatant fluid appeared to be characteristic of pre-neoplastic liver.

The non-carcinogenic dye 2-methyl-4-dimethylaminoazobenzene was a peculiar exception. It greatly increased the protein and riboflavin contents of the large granules. Thus the composition of this fraction changed in a direction opposite that found in tumor tissue. Decreases in the pentosenucleic acid contents of the large and small granules also occurred. As with the other dyes, protein-bound dye was formed in each fraction.

THE EFFECTS OF SEVERAL AMINOAZO DYES ON THE SUCCINOXIDASE OF RAT LIVER. V. R. POTTER, J. M. PRICE (by invitation), E. C. MILLER, and J. A. MILLER. (McArdle Memorial Laboratory, University of Wisconsin, Madison 6, Wis.)

Homogenates of the same livers that were studied by Price *et al.* (see previous abstract) were assayed for the succinoxidase system and the oxalacetic oxidase systems by techniques previously described. The amount of succinoxidase was roughly proportional to the amount of protein and of riboflavin in the mitochondria, in which the enzyme has been shown to be localized. The three constituents were decreased by the carcinogenic dye 3′-methyl-4-dimethylaminoazobenzene and greatly increased by the 2-methyl compound, which is non-carcinogenic. The amount of enzyme did not appear to correlate with any of the other cell constituents.

When rats that had been maintained on the basal diet were placed on the same diet minus protein, the succinoxidase of the livers dropped to approximately the hepatoma level, which was also attained in 4 weeks by feeding the most potent dye.

Since the mass of the mitochondria in terms of protein, riboflavin, and succinoxidase per gram of liver or nuclear material can vary as much as 300 per cent from one experimental condition to another, it appears that the processes of synthesis and destruction of these cytoplasmic particles can proceed at rates that differ from the rates of the corresponding processes in the nucleus or in the cell as a whole. These observations suggest that the particles and the cells may have different rates of multiplication.

FACTORS AFFECTING THE DESTRUCTION OF AZO DYES BY HEPATIC CELLS *IN VITRO*. CHARLES J. KENSLER. (Department of Pharmacology, Cornell University Medical College, New York 21, N.Y.)

Earlier reports from this laboratory have shown that diet influences the ability of rat liver slices to destroy the hepatocarcinogen N,N-dimethyl-p-aminoazobenzene (DMB) and that this activity of liver decreases as their riboflavin concentration falls.

DMB and a series of five related compounds of varying carcinogenic activity have been fed to rats maintained on a casein-dextrose type diet and the riboflavin concentration in the liver and the ability of liver slices to destroy DMB have been determined. The rate at which hepatic cells destroy DMB and eleven related compounds in vitro has also been determined. Methylation of either aromatic ring decreased the rate of destruction. Methylation of the 2′ position produced the greatest decrease. Methylation of the amino group did not greatly decrease the rate of destruction whereas ethylation did. The oral administration of all six azo compounds decreased the ability of liver cells to destroy DMB *in vitro* but the administration of 2-acetylaminofluorene did not. This effect is more closely related to their rate of destruction by liver *in vitro* than to their carcinogenic activity. Livers from fasted rats (39 to 48 hrs.) also showed decreased activity.

The determination of the rate of destruction of the primary and secondary amino compounds was complicated by the fact that 30 to 40 micrograms were lost even though the cells were killed by the immediate addition of alkali. This loss can be prevented by the reducing agents thioglycerol or thioglycolic acid or by keeping the slices cold throughout the homogenization until the benzene extraction of the dye is begun. Heating the tissue (5 min. boiling water) did not prevent the loss which has been found to be associated with the action of KOH on the heat coagulated fraction. In contrast the tertiary amino compounds are destroyed only in fortified homogenates which are inactivated by heat in agreement with the recent report of Mueller and Miller. Cytochrome c greatly inhibits the destruction of DMB in fortified homogenates.

THE CARCINOGENICITY OF CERTAIN FLUORO DERIVATIVES OF 4-DIMETHYLAMINOAZOBENZENE IN THE RAT. J. A. MILLER, R. W. SAPP (by invitation), and E. C. MILLER. (McArdle Memorial Laboratory, University of Wisconsin, Madison 6, Wis.)

The activities of several fluoro and trifluoromethyl derivatives of 4-dimethylaminoazobenzene as hepatic carcinogens for the rat were determined. The 3′- and 4′-fluoro derivatives were more active than 4-dimethylaminoazobenzene while the 2′-fluoro derivative was at least as active as the parent dye. In contrast the 2′-, 3′-, and 4′-trifluoromethyl derivatives of the dye were inactive even after feeding for 8 months. The high activities of the 2′- and 4′-fluoro compounds are of interest since previous work has shown that substitution in these positions with 5 other groups produced compounds with activities considerably below that of the parent compound. Furthermore, the presence of the very strong carbon-fluorine bond in the 2′- and 4′-positions of these active molecules makes it unlikely that a benzidine rearrangement of these dyes occurs *in vivo*. A recheck on the carcinogenicity of 2,4′-diamino-5-dimethylaminobiphenyl, the hypothetical benzidine rearrangement product that might be derived from 4-dimethylaminoazobenzene *in vivo*, showed again that this compound was inactive even after being fed at high levels for 8 months. Two tri-substituted derivatives, 2′, 4′, 6′-trichloro-, and 2′, 4′, 6′-tribromo-4-dimethylaminoazobenzene, were also inactive. These data and those previously reported on structural variations (Miller and Miller. J. Exper. Med., **87**:139, 1948) indicate that steric factors predominate amongst the several factors that probably govern the carcinogenicity of ring-substituted derivatives of 4-dimethylaminoazobenzene.

THE CARCINOGENIC ACTIVITY OF SEVERAL STRUCTURAL ANALOGS OF 2-ACETAMINOFLUORENE IN THE RAT.

E. C. Miller, J. A. Miller, R. B. Sandin (by invitation), and R. K. Brown (by invitation). (McArdle Memorial Laboratory, University of Wisconsin, Madison 6, Wis., and Department of Chemistry, University of Alberta, Edmonton, Alberta, Canada.)

To study some structural features involved in the carcinogenic activity of 2-acetaminofluorene, 0.036 per cent of 2-acetaminofluorene and equimolar levels of 3-acetaminodibenzothiophene, 3-acetaminodibenzothiophene-5-oxide, and 3-acetaminodibenzofuran were fed in a grain diet for 8 months. Each compound induced mammary adenocarcinomas in female rats, but 2-acetaminofluorene and 3-acetaminodibenzothiophene were the most active; 60 per cent of the females fed these compounds had tumors at 4 months. Squamous cell carcinomas arising from or near the ear duct developed in rats fed each compound; the incidences were about 50 per cent for both the male and female rats fed 2-acetaminofluorene and 3-acetaminodibenzothiophene for 8 months. Only 2-acetaminofluorene elicited liver tumors; they were found in 60 per cent of the males fed this compound for 8 months. In another connection 0.105 per cent of 4-dimethylaminodiphenyl was fed in a purified diet for 8 months. Of the 10 males surviving for 8 months 3 developed subcutaneous tumors and 2 had squamous cell carcinomas arising from or near the ear duct.

These data demonstrate the effect of substituents in the central ring on the carcinogenicity of 2-acetaminofluorene. Replacing $-CH_2-$ by $-S-$ does not alter the carcinogenicity of the molecule for either mammary or ear duct tissue but abolishes its activity for the liver. Substitution of $-S-$ or $-O-$ diminishes the activity for the mammary and ear duct tissue and abolishes it for the liver. Omission of the $-CH_2-$ bridge as in 4-dimethylaminodiphenyl still permits the induction of subcutaneous and ear duct tumors.

PRECANCEROUS CHANGES IN THE LIVER PROTEINS OF RATS FED ACETYLAMINOFLUORENE.

A. Clark Griffin, Hyla Cook, and J. Murray Luck (Introduced by Joseph C. Aub). (Department of Chemistry, Stanford University, Cal.)

Albino rats were maintained on purified diets containing 0.04 per cent acetylaminofluorene for a period sufficiently long to induce liver tumors. Animals were sacrificed at approximately one month intervals during this precancerous period and the total nitrogen, phosphorus, desoxyribonucleoprotein, ribonucleoprotein, and riboflavin content of the livers determined. Similar determinations were also made on liver tumors induced by this agent. Parallel observations were made on the serum proteins by the electrophoretic method.

During acetylaminofluorene carcinogenesis there was a decrease in the liver desoxyribonucleoprotein which is in contrast to the azo dye carcinogenesis wherein this fraction increases. The liver concentration of ribonucleoprotein, riboflavin, nitrogen, and phorphorus also decreased as the acetylaminofluorene was fed. Liver tumors resulting from this agent had lower concentrations of desoxyribonucleoprotein, nitrogen and phosphorus than liver tumors induced by the azo dyes. The livers showed a progressive increase in size as the diet containing the acetylaminofluorene was fed.

The dietary acetylaminofluorene resulted in some increase in the β-globulin of the serum, other serum proteins remained relatively normal. This is contrasted to the increase observed in the serum γ-globulin fraction and no apparent effect on the β-globulin component that results from the carcinogenic azo dyes.

HEPATOMAS FOLLOWING INTRAHEPATIC INJECTION OF "MITOCHONDRIA FRACTION" OF CHEMICALLY INDUCED HEPATOMAS.

J. Stasney, K. E. Paschkis, and A. Cantarow. (Jefferson Medical College, Philadelphia 7, Pa.)

Portions of hepatomas induced in 37 rats by feeding 2-acetaminofluorene were homogenized and fractionated by the differential centrifugation technique of Claude, as modified by Hogeboom *et al.* A sample of each hepatoma "mitochondria fraction" was examined for the presence of intact cells or nuclei. None was found. One hundred gm. of normal liver was subjected to the same procedure and the entire "mitochondria fraction" examined for the presence of intact cells or nuclei. None was found.

Various fractions were injected into or implanted in the livers of normal adult rats under ether anesthesia.

Forty-six rats were thus inoculated with the "mitochondria fraction." Others were so inoculated with (a) crystals of acetaminofluorene suspended in plasma, (b) suspensions of intact hepatoma cells, and (c) the fraction removed at the first centrifugation, containing coarse cellular and nuclear particles and perhaps some intact nuclei.

The host animals were sacrificed at varying intervals following inoculation. Typical hepatomas were found, after 29 and 35 days, respectively, at the site of inoculation in 2 of 36 rats receiving the "mitochondrial fraction" that survived longer than 7 days. None was present in any of 48 animals receiving the other materials. Focal necrosis with fibroblastic reaction was frequently present at the inoculation site, especially in animals receiving the "mitochondria fraction." The significance of these observations is discussed.

TUMOR PRODUCTION AND METABOLISM OF AZOTOLUENE, AZOTOLUENE SPLIT PRODUCTS AND ANILINE IN RATS ON VARIOUS DIETS. B. Ekman and J. P. Strömbeck (Introduced by Konrad Dobriner). (Department of Biochemistry and University Clinic, University of Lund, Sweden)

Tumors of the bladder have been produced by feeding rats on a restricted diet described by Bowman and Miller together with 2,3-azotoluene or its split products o- and m-toluidine and o- and m-aminobenzoic acid. The same type of tumors are produced by feeding aniline on the same diet whereas p-aminophenol does not give any tumors. The metabolism of these compounds was studied in rats on various diets and it was observed that with a complete diet when no tumors were obtained, increased amounts of the oxidized excretion products (aminobenzoic acids, aminocresols and p-aminophenol) were found in the urine. It has been possible to influence both the metabolism of the tumorigenic compounds and the tumor frequency by varying the protein and vitamin B (especially riboflavin) content of the diet. The increased output of the oxidized compounds was parallel to an increased synthesis and excretion of ascorbic acid. The role of the ascorbic acid in the metabolism of the compounds studied will be discussed.

ANOMALOUS ACTION OF CROTON OIL AS CARCINOGENIC PROMOTING AGENT. I. Berenblum (by invitation). (National Cancer Institute, Bethesda 14, Md.)

Croton oil, itself non-carcinogenic, can elicit tumors in mouse's skin pre-treated a few times, or even once only, with a carcinogen (Berenblum, 1941, Mottram, 1944). Under such conditions, the total tumor yield is dependent on the effectiveness of the preliminary action of the carcinogen, while the average latent period is a function of the subsequent croton oil treatment. (Berenblum and Shubik, 1947; 1949, *In press.*)

A slight, but significant, difference in latent period was noted between the earlier and later series, both carried out in Oxford, England. The use of different batches of croton oil, in the two series, and the fact that mice from different sources were employed, might both have been responsible for the differences observed.

A further investigation by the author is now in progress at the National Cancer Institute, Bethesda, employing croton oil of yet another source (from this country), and using 5 different inbred strains of mice (C, C3H, dba, C57 brown, and C57 black). These experiments, not yet completed, already indicate a low promoting effect of croton oil (less than 1 per cent tumor yield after 10 weeks of croton oil treatment, as compared to over 30 per cent, after the same interval in previous studies). About 600 mice are being employed in this study.

Possible explanations of these results to be considered are: (a) variation in concentration of the 'promoting factor' in different batches of croton oil; (b) presence of anticarcinogenic substances in some batches of croton oil; (c) influence of strains of mice used; and (d) conditions of maintenance (diet) and housing of animals, etc. These will be discussed.

SOME OBSERVATIONS ON THE BIOCHEMICAL EFFECTS OF BERYLLIUM. Robert S. Grier, Mahlon B. Hoagland, and Margaret Hood (Introduced by Joseph C. Aub). (From the Medical Laboratories of the Collis P. Huntington Memorial Hospital of Harvard University at the Massachusetts General Hospital, Boston, Mass.)

The relative simplicity of the element beryllium as a sarcomagenic and rachitogenic agent and its close periodic relation to magnesium warrant study of the biochemical phenomena involved. Because of the importance of alkaline phosphatase in bone metabolism and of the activating effect of magnesium thereon, the effect of beryllium on several phosphatases has been studied.

Beryllium in small concentrations markedly inhibits the alkaline phosphatase activity of crude extracts of bone, intestine, serum, and sarcoma tissue as well as a relatively purified intestinal phosphatase. Our observations suggest a competitive relationship between beryllium and magnesium to this enzyme. Furthermore, reversal of magnesium activation and beryllium inhibition can be produced by adding citrate to the system.

INDUCTION OF ADENOCARCINOMA AND OTHER LESIONS OF GLANDULAR STOMACH IN RATS BY INTRAMURAL INJECTION OF 20-METHYLCHOLANTHRENE. Harold L. Stewart, William V. Hare (by invitation), Egon Lorenz, and James G. Bennett (by invitation). (National Cancer Institute, Bethesda 14, Md.)

Six-tenths milligram of methylcholanthrene suspended in an aqueous solution of methyl cellulose (Methocel 4000 cps.) was injected at each of two sites into the wall of the glandular stomach of rats of the Osborn-Mendel, Marshal 520, and AXC strains. Of 96 animals which have been autopsied the following lesions were observed: adenocarcinoma, 1; adenoacanthoma, 2; sarcoma, 4; and a massive diverticulum like lesion with atypical proliferation of epithelium, 57. The morpho-

logic characteristics of this latter lesion, its pathogenesis, its possible neoplastic nature, and the results of transplantation are presented. A marked difference was noted in the number of lesions induced depending upon the site of injection, the prepyloric region being the more responsive.

BIOSYNTHESIS OF AMINO ACIDS UNIFORMLY LABELED WITH RADIOACTIVE CARBON, FOR USE IN THE STUDY OF GROWTH. IVAN D. FRANTZ, JR. and HOWARD FEIGELMAN (Introduced by PAUL C. ZAMECNIK). (From the Medical Laboratories of the Collis P. Huntington Memorial Hospital of Harvard University, at Massachusetts General Hospital, Boston, Mass.)

The usefulness of amino acids labeled with radioactive carbon has been demonstrated in a number of laboratories. With a few notable exceptions, most of the experiments have been carried out with the simpler, non-essential amino acids, primarily because of the difficulty of synthesizing the more complicated molecules from the radioactive starting materials available, and the low yields obtainable. It appears that more significant results could be obtained with compounds which the tissues being studied are incapable of synthesizing.

The purpose of the present work was to produce useful quantities of the essential amino acids by biosynthesis. The autotrophic bacterium *Thiobacillus thiooxidans* was grown in an atmosphere of radioactive carbon dioxide. The bacterial proteins were isolated and hydrolyzed, and the amino acids separated by starch column chromatography. Because labeled carbon dioxide was the sole source of carbon, the amino acids were uniformly labeled in all positions. This point was confirmed by comparisons of the specific activities of the various amino acids with each other, with allowance for the number of carbon atoms in each. Sufficient quantities were obtained for further studies of tumors in animals. The specific activity of the products was about 150 microcuries per millimole of carbon. The culture vessel was so arranged that any carbon dioxide not utilized by the bacteria could be recovered.

A STUDY OF THE RELATIVE TOXICITY OF N-IODOACETYL AMINO ACIDS AND OF TISSUE DISTRIBUTION OF RADIOACTIVE ANALOGUES CONTAINING I^{131} IN RELATION TO INHIBITION OF GROWTH OF SARCOMA 37 IN SWISS MICE. ORRIE M. FRIEDMAN and ALEXANDER M. RUTENBURG (Introduced by ARNOLD SELIGMAN). (Department of Chemistry, Harvard University, Cambridge, Mass., Kirstein Laboratory of Surgical Research, Beth Israel Hospital, Boston, and Department of Surgery, Harvard Medical School, Boston, Mass.)

Since toxic substances related to essential metabolites seemed of interest for a study of inhibition of growth of tumors, derivatives of amino acids were prepared which were toxic and which could be readily labelled with a radioactive isotope. N-iodoacetyl derivatives of tryptophane, leucine and phenylalanine have been prepared. The relative toxicity in Swiss mice of these 3 substances and iodoacetamide was determined and the ability of the 4 substances to inhibit the growth of Sarcoma 37 in this test animal was studied. The results have indicated that these substances inhibit the growth of this tumor significantly, to different extents and in a manner apparently unrelated to systemic toxicity.

The radioactive analogues of the three iodoacetyl amino acids and iodoacetamide have been prepared by the use of I^{131}. The concentration of radioactivity and its disappearance from blood, tumor, and liver following intravenous injection of these substances have been determined. Radioactivity has been found in the 3 tissues in significant amounts, the concentration of activity in tumor being consistently greater than in liver and less than in blood. The rate of disappearance of radioactivity from tumor in the case of the 3 amino acid derivatives followed a similar characteristic pattern different from that of iodoacetamide.

A LABILE CALCIUM-RIBONUCLEOPROTEIN COMPLEX IN THE REGION OF THE LIVER CELL CORTEX. T. B. ROSENTHAL and A. I. LANSING (by invitation). (Department of Anatomy, Washington University Medical School, St. Louis, Mo.)

A calcium binding mechanism apparently associated with the cell surface undergoes characteristic changes with age and with some abnormal growth patterns (hyperplasia, cancer). We have suggested that this calcium binding mechanism is related to cellular growth. The experiments here reported add information on the chemical nature and localization of intracellular calcium binding.

By employing radiocalcium as a tracer, pyronin as a marker, and "Celite" as a selective adsorbent in a chromatographic procedure we have shown in homogenates of liver cells that calcium is bound to a nucleoprotein of the ribose type. By employing ribonuclease, both in the chromatographic column and in the cytochemical localization procedure of Brachet, further evidence has been found that the cell cortex is the region of calcium binding.

INITIAL USE OF HIGH ENERGY DEUTERONS AND ALPHA PARTICLES IN CANCER RESEARCH.* C. A. TOBIAS (by invitation), PAUL ROSAHN, HAL ANGER (by invitation), and JOHN H. LAWRENCE. (Division of Medical Physics, Radiation Laboratory, and Department of Physics, University of California, Berkeley, Cal.)

Particle beams of high energy ions produced by accelerators, such as the 184″ cyclotron in Berkeley(1), have several applications in the study of the biological effects caused by irradiation and of tumors. Measurements performed on 190 Mev deuterons and 380 Mev alpha particles indicate: (a) that such beams penetrate in tissue to a depth of approximately 14 and 7 cm respectively, with the ionization distributed overwhelmingly in the main core of the beam, and negligibly in the

* This work has been carried out under the auspices of the A.E.C. See also AECD 2099–A.

fraction scattered out, or in the secondary neutrons produced; (b) the curve of specific ionization has a sharp peak, less than 1 cm from the end of the range. The ratio of peak ionization, to the ionization of the particles as they emerge from the cyclotron, is about 4 : 1 for deuterons and 6 : 1 for alpha particles. In agreement with calculations made by Wilson (2), such beams appear applicable to concentrated depth doses of irradiation. Most of the ionization may be concentrated in a sphere $\frac{1}{2}''$ diameter at any part of the body, while the ionization in the tissues not directly in the path of the beam is reduced to a minimum. The ratio of depth dose to surface dose, and the ability to direct the beam to a local spot, appear to be much superior to any other type of radiation. Some preliminary experiments were carried out by using type "A" mice and Strong transplantable mammary carcinoma. The deuteron beam was passed through the body of the animals with absorbers placed in the beam in such a way that the tumors received the highest specific ionization. Some of the tumors showed complete regression this way, without producing too severe radiation damage to the body of the animals. A more complete study is under way concerning specific physiological and biochemical effects produced by irradiation of the animal body, tumors and specific organs.

1. W. M. Brobeck, E. O. Lawrence, K. R. McKenzie, E. M. McMillan, R. Serber, D. C. Sewell, and R. L. Thornton. The initial performance of the 184″ Cyclotron of the University of California. Physical Review, **71**:1947.
2. R. R. Wilson. Radiological Use of Fast Protons. Radiology, **47**:487, 1946.

ANALYSIS OF LYMPHOMA INDUCTION BY X-RAY IN MICE. George A. Sacher (by invitation), and Austin M. Brues. (Argonne National Laboratory, Chicago, Ill.)

CF-1 female mice 120 days of age were treated with total body X-ray, using single and daily fractionated dosages between 400 and 1200 r. The incidence of lymphoma was determined (grossly at autopsy) and studied as a function of time and treatment. The total cumulative incidence is determined by the size of the dose and the fractionation pattern. When the logarithm of the rate of morbidity is plotted against time after treatment, a set of curves is obtained which is characterized by a peak in morbidity a few months after treatment, with a subsequent approach to the normally rising control morbidity curve. The incidence a year or more after treatment is little in excess of that of controls of the same age. When the morbidity rates are considered as the sum of the normal age incidence and the superimposed carcinogenic response, it is found that the carcinogenic response curve changes in amplitude (but not in form) with alterations in the dosage.

EFFECTS OF IRRADIATION WITH X-RAYS ON MAMMARY TUMOR TRANSPLANTS OBSERVED *IN VIVO*. Ruth M. Merwin, Glenn H. Algire, and Henry S. Kaplan. (National Cancer Institute, Bethesda 14, Md.)

Direct microscopic observations through a mica window have been made of the effects of X-radiation on mammary adenocarcinomas transplanted beneath the skin of strain C3H mice. The tumors were exposed to $2\frac{1}{2}$ to five thousand r, the remainder of the animal being shielded. At the time of treatment the implant had usually been in about a week and was 2 to 8 mm. in diameter. Effects were observed on tumor growth rate, vascular reactions, translucency, appearance of fat cells and acinar structures.

Six hours after irradiation the only reaction noted is enlargement of the tumor. Eighteen to thirty hours after treatment no further increase in size is found although there is sometimes shrinkage from the original size at the higher doses. A general opacity of the tumor tissue is conspicuous but no evidence of vascular damage is observed. During the next few days the tumor decreases in size and the vascularity is greatly reduced as the vessels become much narrower or disappear. Resumption of growth of tumor tissue after irradiation was followed in two instances. The first indication of recovery was the enlargement of blood vessels in scattered areas, followed by the development of translucency and the progressive growth of these foci.

The interpretation of the observed changes is discussed in relation to the role of the vascular changes following irradiation.

HISTOLOGICAL CHANGES PRODUCED BY A SINGLE LARGE INJECTION OF RADIO-PHOSPHORUS (P^{32}) IN ALBINO RATS AND IN C3H MICE. B. Grad and C. E. Stevens (Introduced by C. P. Leblond). (Department of Anatomy, McGill University, Montreal, Canada)

Young male and female rats and adult female C3H mice with spontaneous mammary tumors received a single large dose of radio-phosphorus and were sacrificed 2 hours to 10 days later. The destructive effects of P^{32} are most marked in lymphatic organs and bone marrow, small intestine, granulosa of ovarian follicles, and mammary tumors. Minor changes are noted in stomach and colon and at later intervals in the seminiferous tubules of testis, and hair follicles. The cellular destructions observed in all these organs consist of cloudy swelling and nuclear pyknosis. Nuclear abnormalities such as gigantism are found in recovering cells. In the crypts of the small intestine, massive degeneration and pyknosis of dividing cells results in thinning of the epithelium of crypts and villi, probably because no new cells are supplied from the crypts to balance the continual loss of cells by extrusion normally occurring at villi tips.

The mammary tumors show many scattered pyknotic cells at 24 hours after injection. At 48 hours large blood lakes have formed without much tissue damage. At 10 days most of the tumor is necrotic and liquefied, probably because of interference with the blood supply. Little or no damage is observed in salivary glands, pancreas, liver, genitourinary system, endocrine glands, nervous, muscular, and connective tissue.

Thus, as with X-radiation, the major changes occur in organs with a rapid cell turnover. These organs were previously shown to take up large amounts of radiophosphorus from the blood.

EFFECTS OF CHRONIC IRRADIATION WITH GAMMA RAYS ON MAMMARY TUMOR INCIDENCE IN C3Hb FEMALE MICE. Egon Lorenz, Walter E. Heston, and Allen B. Eschenbrenner. (National Cancer Institute, Bethesda, Md.)

One month old C3Hb (C3H without the milk agent) female mice were exposed to 8.8r of gamma radiation for 8 hours daily and autopsied when in moribund condition. The maximum accumulated dose was approximately 5900 r. Mammary carcinomas were observed in 23 per cent of the animals with a mean tumor age of 15 months and sarcomas at the site of the mammary glands were found in 33 per cent with a mean tumor age of 16 months. Comparing the experimental data on mammary tumor incidence with those of Heston (1) on extensively bred C3Hb mice, the conclusion can be drawn that irradiation hastens the appearance of mammary carcinomas without increasing the incidence above that following extensive breeding. While sarcomas at the site of the mammary gland are rare in breeders, a high incidence of this tumor was found in the irradiated animals. The possible connection of this tumor with granulose cell type tumor of the ovaries will be discussed.

(1) Heston, W. E. Role of Genes and Their Relationship to Extrachromosomal Factors in the Development of Mammary Gland Tumors in Mice. Brit. J. Cancer **2**:87–90, 1948.

LOCAL IRRADIATION AND THE INDUCTION OF LYMPHOID TUMORS IN MICE. Henry S. Kaplan. (Department of Radiology, Stanford University School of Medicine, San Francisco, Cal.)

Whole-body exposure of mice to roentgen rays in adequate dosage has long been known to yield lymphoid tumors. Strain A mice, which are moderately susceptible and strain C57 black mice, which are highly susceptible to whole-body irradiation, have been studied following local exposure. Strain C57 black mice one month of age were treated over the anterior or posterior half of the body with daily doses of 100 r for 10 days. After 17 months, only 2 of 55 mice (4 per cent) irradiated over the upper half, and 1 of 55 mice (2 per cent) irradiated over the lower half of the body have developed lymphoid tumors. In previous experiments the incidence of such tumors following whole-body exposure of young mice of this strain to the same total dose has averaged about 65 per cent at 10 months. No exact data are available for half of this total dose delivered to the whole body, but extrapolation indicates that a significantly higher incidence might be expected than was observed after half-body irradiation.

Larger doses sharply localized to the region of the mediastinum or upper abdomen have produced no lymphoid tumors in either strain, and mice of strain A have also been completely refractory to local treatment over the skull or lower abdomen. These results suggest that shielding of one-half or more of the body from roentgen rays confers a considerable degree of protection against the induction of lymphoid tumors in mice.

TYPES OF TRANSPLANTABLE OVARIAN NEOPLASMS INDUCED BY X-RAYS AND THEIR PATHOGENESIS. T. Bali (by invitation), and J. Furth. (Veterans Administration Hospital, Southwestern Medical College, Dallas, Texas)

Most mice exposed to a single 50 r or more develop ovarian growths. Exposure of ovaries seems essential; irridiation of pituitary region is neither productive nor inhibitory. Prenatal irradiation did not yield ovarian tumors, while irradiation at 1 to 3 days of life did, in most animals. Most mice exposed to 150 r at this age are sterilized, but even those having multiple, seemingly normal pregnancies, eventually developed ovarian tumors. The primary growths are usually complex: granulosa, luteoma, and rarely, angioma and sarcoma-like. Practically all of these are benign, *e.g.*, when killed at 19 to 23 months of age, they were found in the majority of mice of the series irradiated at 1 to 3 days of age.

The common tubular structures were least transplantable in series. Those successfully grafted were exceedingly slow-growing.

The x-ray induced growths seem to possess a greater autonomy than those induced by the Biskind procedure. Many of them appear mere massive hyperplasias. Only granulosa tumors and luteomas secrete hormones, and their mother cells are known to be under hormonal control; the mother cell of the common tubular adenomas and the rare types of neoplasms mentioned are neither hormone-producing, nor under hormonal control.

Several factors can be implicated in the genesis of these tumors: a. Excessive pituitary gonadotrophic stimulation as indicated by the Biskind procedure; b. X-rays seem to exert a specific neoplastic stimulus. as on skin and other organs, non returning to "normal" once irradiated; c. Some local derangement of interdependent structures.

FREE AMINO ACIDS IN NORMAL AND NEOPLASTIC TISSUES AS STUDIED BY PAPER CHROMATOGRAPHY. Eugene Roberts. (Department of Anatomy, Division of Cancer Research, Washington University Medical School, St. Louis, Mo.)

A survey was made of the free amino acids found in alcoholic extracts of freshly excised normal and neoplastic tissues. Two-dimensional chromatograms were made on aliquots corresponding to 75 mg. of fresh tissue after oxidation with H_2O_2 on the paper. Sixteen different normal tissues and samples of squamous cell and mammary carcinomata and sarcoma 37 have been examined. Epidermis showed the highest concentration of free amino acids of any of the tissues studied, a finding consistent with the extremely high level of trichloracetic acid soluble nitrogen. With the exception of epidermis, the malignant tissues studied had greater overall concentrations of detectable constituents than did the nor-

mal tissues. The chief ninhydrin-reactive substances detected in normal tissues were glutamic acid, aspartic acid, glycine, taurine, alanine, serine, glutamine, cystine, valine, and the leucines. Histidine was present in large quantities in brain, in small quantities in epidermis and tumors, and was not detectable in the other tissues. Arginine and lysine were found in appreciable quantities only in extracts of epidermis, while proline and hydroxyproline were detected in the epidermis and tumors, but not in other tissues. Some of the interesting differences observed among normal tissues will be discussed. Sample chromatograms will be shown.

There was a consistent pattern of distribution of free amino acids in the malignant tissues which was different from that of any of the normal tissues studied.

THE RELATIVE METABOLISM *IN VITRO* OF ANALOGOUS MOUSE MAMMARY TUMORS. ANNA GOLDFEDER. (Cancer Research Laboratory, Department of Hospitals, City of New York, and Department of Biology, New York University, New York, N.Y.)

In a previous report ("Growth in Tissue Culture of Analogous Mammary Carcinomas and Their Response to Radiation," presented at the 1948 meeting of the American Association for Cancer Research—in press, Cancer Research), it was demonstrated that two analogous mammary tumors autogenous to homozygous hosts of dba and C3H strains of mice, both histologically diagnosed as adenocarcinomas, differed widely in regard to their rate of growth and radiosensitivity.

The present study is concerned with the relative rates of respiration, aerobic and anaerobic glycolysis of the above mentioned tumors.

Actively growing tumors, free from necrotic portions, were used. For the determination of respiratory changes the Barcroft-Warburg manometric technique was employed; for the determination of lactic acid in the substrate the Friedman-Contonio-Wendel method was used. Results obtained showed the metabolism of these two tumors (dba, C3H) to differ significantly. Thus Q_{O_2} of the mammary tumor of the dba strain averaged 5.6; $Q_G{}^{O_2}$ averaged 25.2; $Q_G{}^{N_2}$ averaged about 75.0 (dry weight, 60 minute basis), while the Q_{O_2} of the C3H mammary tumor averaged 3.8; $Q_G{}^{O_2}$ 8.6; $Q_G{}^{N_2}$ 55.8.

From both sets of experiments (growth and metabolism of the tumors used in this study) it is noted that, although both tumors appear histologically identical, nevertheless their biological and physiological characteristics appear to differ widely. One may infer that the morphological appearance of tumors is not a sufficient basis for their classification.

The significance of the metabolic rate in relation to radiosensitivity of tumors will be discussed.

YOLK SAC CULTIVATED TUMOR TISSUE AND EXPERIMENTS IN TUMOR CHEMOTHERAPY. II. NEUTRAL RED, ETHYL VIOLET, AND JANUS GREEN. ALFRED TAYLOR and IRVING GALINSKY (by invitation). (Biochemical Institute of the University of Texas and the Clayton Foundation for Research, Austin, Texas.)

Cancer tissue cultivated in eggs by the yolk sac method has been found to serve for the rapid testing of possible anti-cancer compounds. Eggs were inoculated with tumor tissue on about the fourth day of incubation. By the twelfth day tumor tissue was well established on the inner wall of the yolk sac. The test compound was injected on the twelfth day of incubation through a tiny perforation in the shell in such manner that the material was deposited between the shell and the chorio-allantoic membranes. The procedure did not increase the egg mortality over that of untreated controls. The experimental eggs and their saline injected controls were harvested 48 hours later on the fourteenth day of incubation and the effect of the test compound evaluated as it affected the weight of the tumor and the chick embryo. The yolk sac tumors were less variable in size and grew much more rapidly than the same tumor cultivated in its normal host, the C3H mouse.

Tests were made with neutral red, ethyl violet and Janus green which have been reported to inhibit cancer growth in mice. Altogether 11 experiments involving more than 300 tumor bearing eggs were completed. Neutral red at the maximum sublethal dose did not affect tumor or embryo growth. Ethyl violet inhibited tumor growth an average of 20 per cent; embryo growth was unaffected. Janus green slightly inhibited the growth of both tumor and embryo.

EFFECT OF FOLIC ACID ANTAGONISTS ON TRANSPLANTED MOUSE LEUKEMIA. ARTHUR KIRSCHBAUM, SISTER TERESITA JUDD (by invitation), NANCY GEISSE (by invitation), and LEO M. MEYER (Department of Anatomy, University of Minnesota Medical School, Minneapolis, Minn., and Department of Therapeutics, New York University Medical School, New York, N.Y.)

The effect of 9 folic acid antagonists (including aminopterin), teropterin, and folic acid on the survival time in 2 lines of myeloid and one line of lymphoid (?) transmitted leukemia was determined. The compounds were administered in maximum tolerable daily doses or doses comparable to those used clinically; treatment was begun on the day following transfer of the disease. Animals of the same transfer generations which received (a) either no treatment, or (b) Fowler's solution, or (c) urethane served as controls. The lines of leukemia originated in the F strain. Routine transfer was accomplished by intraperitoneal injection of one to five million splenic cells suspended in isotonic saline.

Survival was consistently but only slightly prolonged (compared to untreated mice) by the administration of the folic acid antagonists in the 2 lines of myeloid leukemia studied; in none of 90 was the development of the disease completely inhibited. Twenty-four of 36 animals receiving Fowler's solution (0.1 mg. daily) did not become leukemic, and in the others survival was longer than in mice receiving folic acid antagonists, teropterin, or folic acid. Urethane-treated controls also survived longer than test animals. In the third line where the cells were undifferentiated, and the type of leukemia may be open to question, and in 2 additional transfer lines of myeloid leukemia, similar results were

obtained. Neither folic acid nor teropterin accelerated the development of the transplanted leukemias.

THE EFFECT OF REGENERATION ON THE RATE OF PROTEIN SYNTHESIS AND DEGRADATION IN RAT LIVER. NANCY L. R. BUCHER and ROBERT B. LOFTFIELD, and IVAN D. FRANTZ, JR. (by invitation). (Medical Laboratories of the Collis P. Huntington Memorial Hospital of Harvard University at the Massachusetts General Hospital, Boston, Mass.)

The capacity of tissue to grow is intimately related to its ability to build proteins. A constant turnover of protein occurs under resting conditions. When additional protein is formed there must be either increased rate of synthesis, decreased rate of degradation, or both.

Using amino acids labeled with C^{14} we have investigated protein turnover in regenerating rat liver, a normal tissue that grows faster than most neoplasms. *In vitro* studies showed that slices of regenerating liver incorporated tagged alanine into protein at a maximum of 3.5 times the normal rate. *In vivo* experiments, using both alanine and glycine, demonstrated an increase of 1.5 to 2 times normal.

In degradation studies radioactivity was established in liver protein by administering labeled glycine. Inactive glycine was then force-fed to minimize the reutilization of tagged molecules derived from breakdown products in the metabolic pool. The half-life of activity in the protein of resting liver was 3 days, whereas in non-glycine-fed rats a value of 5.5 days was obtained.

During growth, allowance must be made for dilution of tagged protein by new protein added. Regenerating liver provided a unique opportunity to determine this, since the amount of tissue left behind following partial hepatectomy could readily be calculated. When glycine was force-fed, the protein of regenerating liver was found to have a half-life of 5.5 to 11.5 days; without glycine radioactivity diminished very slowly.

These findings suggest that the rate of protein synthesis increases and possibly that of protein degradation decreases during hepatic regeneration.

THE INFLUENCE OF NORMAL SERUM AND HODGKIN'S SERUM ON CELLULAR GROWTH AND MORPHOLOGY IN TISSUE CULTURE. MARGARET S. REIMAN (by invitation), and HERMAN A. HOSTER. (The Ohio State University College of Medicine, Columbus, Ohio)

A study of the effect of normal serum and Hodgkin's serum on normal and diseased cells in tissue culture has been undertaken. An attempt has been made to compare the morphologic changes, which occur in normal cells and diseased cells as a result of the presence of Hodgkin's serum in the nutrient milieu, with the morphologic characteristics observed in the 3 types of Hodgkin's explant growth in the presence of normal serum.

The present study is based on 55 tissue specimens and 1500 explants cultured in the roller tube without transplantation for 3 to 20 weeks (average 6 weeks). All observations reported were made on living cells at 100 and 500 magnifications. Alterations in morphology and cellular growth patterns observed in cells nourished with Hodgkin's serum occurred following the use of most but not all sera samples studied.

Three general types of growth were observed in Hodgkin's lymph node cultures. These have been tentatively classified as follows: (a) the granule-containing macrophage and/or reticulum cell type, (b) the granule-free asyncytial round or elongated cell type, and (c) the fibroblast type. Alterations in the morphology and cellular growth pattern observed both in cells nourished with Hodgkin's serum and in Hodgkin's tissue growth types a, b, and c will be illustrated.

DIET AND AZO DYE TUMORS: EFFECT OF DIET DURING PERIODS WHEN THE DYE IS NOT FED. C. C. CLAYTON (by invitation), and C. A. BAUMANN. (Department of Biochemistry, University of Wisconsin, Madison 6, Wis.)

Rats fed m′ methyl p-dimethylaminoazobenzene (m′DAB) develop hepatic tumors even though the feeding of the dye is interrupted for periods of 4 to 12 weeks. In a typical experiment the dye is incorporated into a standard synthetic ration and fed for 4 weeks, one of several dye-free diets is fed during the next 4 weeks, the feeding of the dye in the synthetic ration is repeated for 4 weeks, and during the final 8 weeks the dye-free synthetic ration is fed. Under this arrangement the number of tumors formed depends upon the diet fed during the period of interruption.

Diets high in fat or in methionine failed to alter tumor formation. Caloric restriction during the period of interruption not only failed to diminish tumor incidence, but actually appeared to enhance it. Diets high in riboflavin and protein decreased the number of tumors formed; while a diet low in labile methyl groups and containing nicotinamide increased the numbers of tumors. The results suggest that some of the so-called effects of diet on tumor formation may be exerted on the animal itself rather than on an altered metabolism of carcinogen.

In contrast to the effects of diet observed during the period of interruption no very pronounced effects of diet were noted when the carcinogen was fed continuously for 8 weeks and the dietary variation introduced subsequently.

BLOOD PRESSURE MEASUREMENTS IN TRANSPLANTED TUMORS OF UNANESTHETIZED MICE. GLENN H. ALGIRE. (National Cancer Institute, Bethesda, Md.)

The transparent chamber technique as adapted to a skin flap in mice makes accessible for microscopic observation a layer of subcutaneous and muscular tissue approximately 0.5 mm. thick, and having a surface area of 150 sq. mm. Microscopic observations of normal and neoplastic tissue transplants within the chamber may be made at magnifications up to 500 ×.

An indirect method has been devised for blood pressure measurements of any vascular component within

the chamber during direct microscopic examination by transmitted light. Measurements may be made on the same vessel intermittently throughout the day, and repeatedly for the duration of the preparation (approximately 30 days). The apparatus consists of a mercury sphygmomanometer system and air reservoir connected to a glass tube having a flexible, translucent membrane tied loosely across the end. A micromanipulator is used to bring the membrane into contact with the under surface of the skin. Pressure applied using the sphygmomanometer bulb results in slight bulging of the membrane. As the entire field is visualized under the microscope one can obtain a measure of the arterial, systolic and diastolic pressures, and venous pressures. The pulse wave can be seen at maximum amplitude approximately midway between systolic and diastolic pressures.

Parallel observations and measurements have been made in vessels of both normal and neoplastic tissues of such correlated vascular phenomena as changes in caliber of vessels, rates of flow, vasomotion, intravascular agglutination. Data are presented showing relationships of host and tumor blood pressure and vascular supply under various experimental conditions leading to tumor damage.

VIRAL HEPATITIS AND HODGKIN'S DISEASE. HERMAN A. HOSTER and ROBERT P. ZANES, JR. (by invitation). (The Ohio State University College of Medicine, Columbus, Ohio.)

The present study is concerned with the effect of an infectious disease, viral hepatitis, on the course of Hodgkin's disease. Reports dealing with the favorable influence of viral hepatitis on other disease entities have been limited to rheumatoid arthritis (1). More recent accounts have ascribed this phenomenon to "non-specific disturbances of liver function" and not to the presence of an infectious agent.

The material presented is based on two cases observed for 5 and 6 years following the onset of hepatitis, one case which terminated fatally with the onset of jaundice, and 21 cases observed for 3 to 8 months after inoculation with viral hepatitis containing sera and tissue extracts.

The evaluation of results presented is based principally on changes in hematologic equilibrium and on alterations in the clinical status of the patient. Unusual gross and microscopic findings in the single death resulting from hepatitis will be presented.

(1) STILL, G. F. Tr. Roy. Med.-Chir. Soc., **80**:52, 1897.

MUCOPOLYSACCHARIDES AND MUCOLYTIC ENZYMES *IN VITRO*. NORMA E. SHIFRIN (by invitation), and WILLIAM L. SIMPSON. (Detroit Institute of Cancer Research, Detroit 1, Mich.)

Conflicting data on the occurrence of mucolytic enzymes in extracts of malignant tumors and in the effects of such enzymes on the invasive properties of malignant cells indicate the need for new and more carefully controlled studies on the relationship of these substances to malignancy. Attempts have been made to study these complex polysaccharides and related enzymes by tissue culture methods.

Preliminary experiments have been carried out to determine whether cells in culture can utilize or can acquire the ability to utilize mucopolysaccharides. The effect of varying concentrations of purified hyaluronic acid (HA) was tested on hanging-drop cultures made up of 1 drop of chicken plasma and 1 drop of 1 HA : 1 chick embryo extract were prepared to contain concentrations of 0.210 mg., 0.072 mg., and 0.021 mg. per culture.

In 7 out of 10 experiments using the above concentrations, growth inhibition seemed to be proportional to the amount of HA present. That is, the most growth occurred in the control groups, the least growth in the 0.210 mg. concentration groups. All of the cultures raised on concentrations of 0.210 mg. and 0.072 mg. died within 3 to 5 days after the last experiment. The cultures growing in a concentration of 0.021 mg. survived for as long as did the controls. The 10 experiments were carried out in 22 days. Attempts to repeat and extend these studies using the roller tube method of tissue culture are now being carried on.

A NEW REAGENT FOR THE HISTOCHEMICAL DEMONSTRATION OF ACTIVE CARBONYL GROUPS. ARNOLD M. SELIGMAN and RIVKA ASHBEL (by invitation). (Department of Surgery, Beth Israel Hospital, Boston, and Harvard Medical School, Boston, Mass.)

A hydrazide containing a naphthol nucleus has been synthesized. After the hydrazide has reacted with active carbonyl groups the naphthol moiety can be coupled with diazonium compounds to form colored pigments. Utilizing this reagent ketosteroid in adrenal cortex was converted to a deep blue pigment. Cells in the corpus luteum of ovary contain sufficient progesterone to give a deep blue pigment. *In vitro* tests showed that the reagent reacts with 3, 17, and 20 keto groups of steroid but not with 11-keto steroid. It may prove useful in urine analysis. The reagent may also be used for demonstrating aldehyde produced in collagenous tissue with periodic acid.

A rich source of carbonyl groups has been found in the non-lipoid component of the white matter of the central nervous system and peripheral nerves.

DISTRIBUTION STUDIES WITH NITROGEN MUSTARD CONTAINING RADIOACTIVE IODINE. ARNOLD M. SELIGMAN and ALEXANDER M. RUTENBURG, and ORRIE M. FRIEDMAN (by invitation). (Department of Surgery, Beth Israel Hospital, Boston, and Harvard Medical School, Boston, Mass.)

Diethyl (β-iodoethyl) amine hydrochloride and methyl bis-(β-iodoethyl) amine hydrochloride have been prepared with radioactive iodine (I^{131}). These compounds are a little more toxic than their β-chloroethyl homologues. Following intravenous injection in mice, rats, and humans, the rate of disappearance of radioactivity from the blood was similar to the rate of disappearance of radioactivity following the injection of an equivalent quantity of radioactive sodium iodide in the first 4 to 8 hours. After this period, a small quan-

tity of radioactivity persisted in the blood in the case of the mustards. Tissue analysis showed greatest persistence of radioactivity in lung, blood, and lymphoid tissue. Tumors contained no more radioactivity than other tissues. Following injection of the mustards ionic halogen is rapidly liberated in the blood. Only a few percent of the mustard reaches the tissues (except lung and blood) in uncyclized form.

FURTHER STUDIES ON THE METABOLISM OF 4-DIMETHYLAMINOAZOBENZENE BY RAT LIVER HOMOGENATE. G. C. Mueller and J. A. Miller. (McArdle Memorial Laboratory, University of Wisconsin, Madison 6, Wis.)

Rat liver homogenates fortified with diphosphopyridine nucleotide, nicotinamide, magnesium ion, and hexose diphosphate metabolized the hepatic carcinogen 4-dimethylaminoazobenzene in several ways. Thus small quantities of the demethylated derivatives 4-monomethylaminoazobenzene and 4-aminoazobenzene and a new metabolite, 4′-hydroxy-4-dimethylaminoazobenzene, were isolated from the reaction mixture. However, more dye disappeared from the reaction mixture than could be accounted for by the azo metabolites found. Presumably cleavage at the azo linkage occurred. The enzymes involved in these reactions appeared to be concentrated largely in the microsome and soluble protein fractions of the liver.

An additional factor involved in the metabolism of the dye was discovered when it was found that the rat liver homogenates lost the ability to metabolize the dye when incubated at 37° C. for 5 minutes. Fortification in the manner described above did not restore the activity but the preparation was reactivated when a liver kochsaft or a concentrate of triphosphopyridine nucleotide (TPN) was added. The probable identity of this factor with TPN was indicated by the observation that the reactivation of the incubated homogenates by the TPN concentrates was proportional to the content of TPN in these concentrates.

PRELIMINARY SCREENING OF 1000 CHEMICAL AGENTS FOR POTENCY IN PRODUCING DAMAGE IN SARCOMA 37. M. J. Shear and V. Downing, and J. L. Hartwell (by invitation), J. Leiter, R. C. MacCardle, and A. Perrault and D. L. Vivian (by invitation). (Chemotherapy Section, National Cancer Institute, Bethesda, Md.)

More than 1,000 chemical agents have been screened against Sarcoma 37 *in vivo* in a preliminary fashion, *viz.*: a single maximum tolerated dose of each compound was injected subcutaneously into mice with week old implants of tumor; the animals were sacrificed at 8, 24, and 48 hours after injection. Gross and microscopic observations were made. The criteria employed in estimating damage induced in the tumors were the same as described in previous years.

In addition to the results presented in the accompanying abstracts, data obtained for other classes of compounds were as follows:

Chemical class	No. of compounds examined	No. yielding positive results
Quaternary ammonium salts	283	13
Acridines	41	7
Phenazines	30	3
Sulfonamides	12	0
Unsaturated ketones	34	3
Isoquinolines	17	0
Quinones	30	8
Stilbenes	17	5
Alkaloids	60	4
Di- and tri-phenyl methanes	40	2
Amidines and guanidines	24	1
Phenyl-C-C-C-phenyl N N	25	0

EFFECT OF PELTATINS ON CYTOCHROME OXIDASE ACTIVITY OF MOUSE TUMORS AND ORGANS. V. S. Waravdekar (by invitation), and J. Leiter. (Chemotherapy Section, National Cancer Institute, Bethesda, Md.)

The cytochrome oxidase activity of 6 day old implants of Sarcoma 37 was determined in tumor homogenates obtained from mice sacrificed 1, 2, 4, and 8 hours after a single subcutaneous injection of 20 micrograms of alpha- or beta-peltatin per gram of body weight. A rapid reduction of enzyme activity to about 40 per cent of the activity of tumor homogenates from untreated animals occurred within 8 hours after injection. This effect paralleled closely the tumor damage seen histologically (MacCardle) in the same tumors. Injection of 500 to 1000 micrograms per gram of an inactive diastereoisomer and of an acetylated derivative of alpha-peltatin produced reductions in enzyme activity which corresponded roughly to the cytotoxic activity of these derivatives as observed histologically in adjacent portions of the same tumors.

The enzyme activity of liver, spleen, and kidney from the same animals showed little or no difference from the activity of organs from untreated tumor bearing control mice. Even lethal doses of 100 micrograms of the peltatins in mice not bearing tumors produced only slight lowering of the enzyme activity in these organs.

In experiments in which homogenates of Sarcoma 37, liver and spleen were incubated *in vitro* with the peltatins, only slight reduction of cytochrome oxidase activity was observed.

One hundred mg. per kilogram doses of the alpha-peltatin injected intravenously in rabbits likewise did not give a pronounced lowering of enzyme activity in liver and kidney.

ACTION ON SARCOMA 37 OF COMPOUNDS RELATED TO PODOPHYLLOTOXIN AND TO ALPHA- AND BETA-PELTATIN. J. Leiter and J. L. Hartwell (by invitation). (Chemotherapy Section, National Cancer Institute, Bethesda, Md.)

Alpha- and beta-peltatin were converted into their diastereoisomers by treatment with alkali. The biologi-

cal activity, both as regards acute toxicity and effect on Sarcoma 37, following subcutaneous injection, was greatly reduced by this treatment. Doses of the new isomers up to 1000 micrograms per gram of body weight produced no deaths in mice and induced no effects in the tumors other than occasional mitotic arrest. In contrast, the parent substances produced grossly visible damage in tumors of various types at doses of two to five micrograms per gram of body weight.

Acetates, methyl and ethyl ethers of the peltatins and their diastereoisomers were prepared; the diastereoisomers and their derivatives had low biological activity whereas the peltatins and their analogous derivatives had high activity. The acetylated derivatives of podophyllotoxin and of its relatively inactive isomer picropodophyllin had activities similar to those of the compounds from which they were derived.

Several benzoic acid derivatives, which represent oxidation residues of podophyllotoxin and of the peltatins, were found inactive against Sarcoma 37. A reduction product of podophyllotoxin was likewise found to be inactive under the conditions of these experiments.

A number of compounds structurally related to podophyllotoxin showed little or no activity at doses 500 times the minimum effective dose of podophyllotoxin. Among these were: conidendrin, dimethyl and diacetyl conidendrin, iso-olivil, isolariciresinol, hinokinin, matairesinol and its methyl ether, asarinin, guaiaretic acid, nordihydroguaiaretic acid, lariciresinol, sodium pinoresinol and its methyl ether, and sesamin.

TOXICITY AND HEMATOLOGIC CHANGES PRODUCED BY ALPHA-PELTATIN, BETA-PELTATIN AND PODOPHYLLOTOXIN. Ezra M. Greenspan (by invitation), and J. Leiter. (Chemotherapy Section, National Cancer Institute, Bethesda, Md.)

The maximum tolerated single dose (MTD) of podophyllotoxin, alpha-peltatin, and beta-peltatin in normal mice was approximately 30, 40, and 50 micrograms/gram, respectively. A single dose of 5 micrograms/gram regularly induced damage in several different types of transplanted mouse tumors. No gross toxicity or weight loss was observed in non-tumor bearing mice after repeated daily injections, for several weeks, of 5 micrograms/gram of alpha- or beta-peltatin.

The MTD for a single injection in rats without tumors was 5 and 10 micrograms/gram for alpha- and beta-peltatin, respectively. In rabbits the MTD for alpha- was 5 mg./kg., for beta-, >20 mg./kg. In dogs the maximum dose tolerated was 0.5–1.0 mg./kg. for alpha-peltatin, 1–2 mg./kg. for podophyllotoxin, and 2–4 mg./kg. for the beta- compound.

The subcutaneous, intramuscular, and intravenous routes of administration were used in mice, rats, and rabbits; dogs received the drug intravenously. Pronounced differences in MTD were not noted among the several routes of administration.

After parenteral injection of lethal or sublethal doses, toxic phenomena were observed in 2 to 6 hours. These included: diarrhea, salivation and emesis (dogs), respiratory and central nervous system depression, muscular paralysis, and a definite pattern of response in the formed elements of the peripheral blood and bone marrow. No evidence of renal or hepatic toxicity was observed at these dose levels. Leucopenia developed ½ to 2 hours after injection. This was followed by leucocytosis (with degenerating neutrophiles and lymphocytes), normoblastosis, and a terminal leucopenia 24 to 48 hours after injection. Degeneration and aplasia of bone marrow were observed at lethal doses.

RELATIVE TOXICITY TO NORMAL AND TUMOR BEARING MICE OF CERTAIN AROMATIC TRIVALENT ARSENICALS WHICH INDUCE HISTOLOGICAL DAMAGE IN SARCOMA 37. Lyle V. Beck, and Adrian Perrault, and Joyce Gillespie (by invitation). (Chemotherapy Section, National Cancer Institute, Bethesda, Md.)

Of 39 pentavalent arsenicals tested (both aliphatic and aromatic), only sodium cacodylate produced gross and histological damage to Sarcoma 37 at maximum tolerated doses (MTD). On the other hand, of 24 trivalent aromatic arsenicals tested at MTD, or less, 6 produced gross and histological damage, and 5 others produced early cell damage and/or aberrant mitotic figures.

Certain of these trivalent arsenicals have been found to be less toxic to CAF_1 mice bearing week-old implants of Sarcoma 37 than to normal CAF_1 mice of the same sex, age, and body weight. For example, 3-amino-4-hydroxyphenyl dichlorarsine hydrochloride (Clorarsen) was found to have a 48 hour LD_{50} value of 11.4 ± S.E. 0.55 micrograms of arsenic per gram in normal CAF_1 female mice, whereas the LD_{50} value for such female mice with 6 day tumors was 16.9 ± 0.9 micrograms of arsenic per gram. Similar results were obtained in two additional experiments with this same compound, and in one or more experiments with the following: 3-acetamino-4-hydroxyphenyl arsenoxide; 3-nitrophenyl arsenoxide; 3,3′-diamino-6,6′-dimethoxyarsenobenzene-N,N′-dimethylene disodium sulfonate; and 3,3′-diamino-6,6′-dimethylarsenobenzene-N,N′-dimethylene disodium sulfonate. Data obtained so far indicate that 3-methoxyphenyl arsenoxide and 3-amino-4-hydroxyphenyl arsenoxide (mapharsen) have the same toxicity in normal as in Sarcoma 37 bearing female CAF_1 mice. The significance of these findings remains to be ascertained.

THE BACTERIAL CELL OF *S. MARCESCENS* AS A SOURCE OF TUMOR-NECROTIZING POLYSACCHARIDE. Adrian Perrault (by invitation), and M. J. Shear. (Chemotherapy Section, National Cancer Institute, Bethesda, Md.)

The bacterial cells, grown in a simple medium of inorganic salts and glucose, were removed from cultures of *S. marcescens* in a refrigerated Sharples supercentrifuge. A larger amount of tumor-necrotizing polysaccharide was obtained from the cells than from the filtrates of the same cultures. The bacterial paste sedimented in the centrifuge was diluted with 1 ml. of buffered saline for

each gram of material, and 0.2 M trichloracetic acid was added in a volume sufficient to bring the final concentration of this acid to 0.1 M. Solid phenol was added to give a 5 per cent concentration. This mixture was shaken to a uniform milky suspension and placed in a refrigerator until sufficient quantities were accumulated for large scale fractionation.

The collected suspensions were centrifuged and the sediment discarded. One volume of hexane (boiling range 60 to 70° C.) and three volumes of absolute ethyl alcohol were added. After stirring 10 to 15 minutes a thick gel rose to the surface. The bottom layer was siphoned and discarded. The gel was treated with water, and NaOH was added with the pH kept below 10. From then on the fractionation paralleled the method used in isolating the tumor-necrotizing polysaccharide from the filtrates of the same cultures.

Bioassays in mice bearing Sarcoma 37 showed a tumor-necrotizing activity comparable to the polysaccharide obtained from the filtrates. A positive reaction for carbohydrates was obtained with Dreywood's anthrone reagent and with the Molish test. Benedict's solution was not reduced. Micro-Kjeldahl analysis gave a total nitrogen content of 1.6 per cent.

A STUDY OF THE HISTOGENESIS OF SARCOMA 37 IMPLANTS. C. H. U. Chu (by invitation) and R. C. MacCardle. (Chemotherapy Section, National Cancer Institute, Bethesda, Md.)

Intramuscular transplants of Sarcoma 37 from the right hind leg were introduced subcutaneously into normal mice. Muscle and connective tissue from the left hind leg of tumor-bearing and non-tumor-bearing litter mates were transplanted also subcutaneously to serve as controls. Serial sections were made to study the fate of such implants and the tissue reactions of the hosts from periods of 2 hours to 12 days after transplantation. Results seem to indicate that prior to vascularization (about the fourth day), tumor cells became necrotic. At the periphery of the necrotic implant, giant cells are invariably observed, the origin of which is not yet determined.

EXFOLIATIVE CYTOLOGY OF THE ORAL CAVITY. Paul W. Montgomery (Introduced by Emmerich von Haam). (Department of Pathology, Ohio State University, Columbus, Ohio)

The exfoliative cytology of the oral cavity has been studied in order to investigate its usefulness for diagnostic purposes. By examining a large number of healthy individuals the normal patterns for the various regions of the mouth and for the various age and sex groups have been established. Statistical analysis of the figures obtained by counting the various cell types has proven the statistical significance of some of the differences observed. Various pathological conditions of the oral cavity, including malignant tumors, were studied. It could be shown that the methods of exfoliative cytology possess a limited value for the recognition of oral pathology.

DISCUSSION OF THE ELECTRONIC THEORY OF CARCINOGENESIS. A. R. T. Denues (Introduced by William L. Simpson). (Detroit Institute of Cancer Research, Detroit, Mich.)

The unique theoretical position of the thesis of Alberte Pullman and others, proposing correlation of electronic structure of chemical carcinogens with their biological potency, appears to justify more attention than is apparent in the literature. The results of studies, made with the help of specialists, of the validity of the general treatment are, therefore, presented together with some analyses of the trends in recent publications. Collectively, these seem to indicate that one is not compelled, on either theoretical or experimental grounds, to attach great cogency to this theory at present. However, in view of the fundamental nature of the interest and of its possible promise of a theoretical unification of carcinogenic mechanisms, work to determine the general validity of the proposal is to be encouraged. The orderly development of the theory, with biological testing of its predictions, as currently pursued in Paris, offers some hope of clarifying its importance. Other evidence may come from indirect experiments, such as determinations of relative carcinogenicities of hydrocarbon isologs with critical substitutions of deuterium for protium, to affect local kinetics without affecting the dominant parameter involving electron distribution.

COMPLEMENT FIXATION IN ANIMAL NEOPLASIA. I. A STUDY OF TECHNICS FOR MEASUREMENT OF THE REACTION IN RABBIT SERUM WITH SPECIAL REFERENCE TO THE TEMPERATURE OF INACTIVATION. Helen Thornton, Lester D. Ellerbrook, and Mark Rhees (by invitation), and Stuart W. Lippincott. (Department of Pathology, School of Medicine, University of Washington and the Cancer Control Division, National Cancer Institute, Bethesda, Md.)

The extent of complement fixation upon the admixture of antigen and inactivated serum of New Zealand white rabbits bearing the Brown-Pearce carcinoma has been measured by the addition of graduated known amounts of complement to identical portions of the other reagents. Volumetric measurements of the amount of complement required were made at the endpoint of 50 per cent hemolysis. As a standard of reference for future antigen preparations the antigen employed was a centrifuged saline extract of the neoplasm.

When the sera were inactivated for 30 minutes at temperatures decreasing step-wise from 70° C. to 53° C. the amount of complement required for 50 per cent hemolysis in tests of 1 : 5 or 1 : 10 dilutions of fresh or previously frozen positive sera increased markedly and then decreased. Similar results were obtained with these sera in the absence of antigen although to a less marked degree. Fresh and frozen normal sera on the other hand tended to require slowly increasing amounts of complement although some of the fresh sera again required smaller amounts of complement when inactivated at 53° C. The absolute temperature difference

tended to be less marked with increased dilution of the sera.

These variations with the temperature of inactivation must be taken into account in the evaluation of the results of the tests.

THE EFFECT OF FOLIC ACID AND ANTI-FOLIC COMPOUNDS ON THE GROWTH OF CARCINOMA, SARCOMA, OSTEOGENIC SARCOMA, LYMPHOSARCOMA, AND MELANOMA IN ANIMALS. KANEMATSU SUGIURA and C. CHESTER STOCK. (Division of Experimental Chemotherapy, The Sloan-Kettering Institute for Cancer Research, New York, N.Y.)

An extensive study has been made on the effect of pteroyl glutamic acid (folic acid), 4-amino-pteroyl glutamic acid (Aminopterin), 4-amino-N_{10}-methyl pteroyl glutamic acid (A-Methopterin), 4-amino-pteroyl aspartic acid (Amino-An-Fol), and 2,6-diamino purine on the growth of Sarcoma 180, mammary adenocarcinoma EO 771, Harding-Passey melanoma, Wagner osteogenic sarcoma and Patterson lymphosarcoma in mice and Sarcoma R 39 and Flexner-Jobling carcinoma in rats. Subcutaneous inoculations of tumors into young animals were carried out by the usual trocar method. In general, the first intraperitoneal injection of compounds was given 1 to 7 days after tumor transplantation and injection was continued for 7 to 14 days.

The daily doses of 50 mg. per kg. of folic acid had no effect upon the growth of Sarcoma 180, adenocarcinoma EO 771, Harding-Passey melanoma, Wagner osteogenic sarcoma, Patterson lymphosarcoma, Sarcoma R 39 and Flexner-Jobling carcinoma in animals. Diopterin (100 mg./kg.) and Teropterin (400 mg./kg.) had no inhibitory effect on Sarcoma 180 and Sarcoma R 39. The daily doses of 0.25 mg. per kg. of Aminopterin had destructive effect on rat sarcoma, marked inhibitory effect on Sarcoma 180 and mouse lymphosarcoma, slight inhibitory effect on mouse adenocarcinoma and melanoma, but no effect on mouse osteogenic sarcoma and rat carcinoma. The daily doses of 1.5 to 2.0 mg. per kg. of A-Methopterin had destructive effect on rat sarcoma, marked inhibition on Sarcoma 180 and lymphosarcoma, slight inhibition on mouse adenocarcinoma and melanoma, but no effect on mouse osteogenic sarcoma and rat carcinoma. The daily doses of 45 to 50 mg. per kg. of Amino-An-Fol had destructive effect on mouse lymphosarcoma and rat sarcoma, marked inhibition on Sarcoma 180, slight inhibition on mouse adenocarcinoma, but no effect on mouse melanoma, mouse osteogenic sarcoma and rat carcinoma. The daily doses of 60 to 70 mg. per kg. of 2,6-diamino purine had marked inhibitory effect on rat sarcoma, but had no effect on Sarcoma 180, mouse adenocarcinoma and osteogenic sarcoma.

At effective levels, anti-folic compounds mentioned were toxic to the hosts, causing weight loss and many deaths. These animals showed intense diarrhea, marked reduction in the size of the spleen and number of erythrocytes in bone marrow.

Aminopterin and A-Methopterin produced neither an inhibitory nor curative effect upon spontaneous breast cancers in mice.

FLUORESCENCE STUDIES OF CARCINOGENS IN RAT'S SKIN. PERIHAN CAMBEL (by invitation). (Department of Anatomy, Division of Cancer Research, Washington University Medical School, St. Louis, Mo.)

The carcinogens, 20-methylcholanthrene, and 9,10-dimethyl-1,2 benzanthracene were applied in 0.6 benzene solutions to the interscapular shaved skins of adult rats and their subsequent distribution was observed by fluorescence microscopy. Both carcinogens enter the sebaceous glands as Simpson and Cramer have reported in mice. However, even after 18 paintings the sebaceous glands of these rats persist, which is in sharp contrast to their early destruction in mice. This, and other differences between the fate of the carcinogens in these two species, will be discussed as possible factors in their differing response by cancer production.

THE EFFECT OF TUMORS ON ANTIBODY LEVELS IN MICE. D. R. A. WHARTON (Introduced by H. J. CREECH). (Lankenau Hospital Research Institute and The Institute for Cancer Research, Philadelphia 30, Pa.)

The effect of tumors on antibody levels resulting from injection of *Serratia marcescens* polysaccharide and other antigens has been studied in mice bearing spontaneous and transplanted tumors. Lower agglutinin levels were found in sera of Sarcoma 37 and Sarcoma 180-transplanted Swiss mice, than in sera of similarly treated normals. This might be due to: the tumor or the toxic polysaccharide acting upon the antibody-forming mechanism, or the destructive or inhibitory action of some substance elaborated by the tumor, or induced by it, which acts upon the antibody. Tumor-bearing mice injected with anti-polysaccharide mouse serum 4 days previously showed lower agglutinin levels than similarly treated controls. Apart from whatever action the tumor or polysaccharide might have upon the antibody-forming mechanism, the presence of the tumor appears responsible for development in mice of a substance destructive or inhibitory to antibody.

This substance was not demonstrable in: C3H or Swiss mice bearing spontaneous mammary tumors; a transplantable mammary carcinoma up to fifth transplant generation; a transplantable methylcholanthrene-induced fibrosarcoma. However, Barrett's C3HBa showed definite effect in the forty-ninth to fifty-third transplant generations.

Antipolysaccharide rabbit serum passed through Sarcoma 37 and normal mice showed no difference in titre. The destructive or inhibitory action produced in mice bearing certain tumors seems specific for mouse antibodies and not for rabbit antibodies.

Mice in which tumors have regressed yield antibody titres comparable with those of normal mice. Antibody-destroying, or inhibiting properties seem to disappear when tumors regress.

THE FLUORESCENT FRACTION OF HORSE SMEGMA AND THE POSSIBLE ROLE OF TRITERPENOIDS OF SEBUM-PRODUCING ORGANS IN CARCINOGENESIS. H. Sobel and A. Plaut (by invitation). (Laboratories of Beth Israel Hospital, New York, N.Y.)

The non-saponifiable fraction of horse smegma which had previously been shown to be carcinogenic (Plaut and Kohn-Speyer) was separated into a fluorescent hydrocarbon-containing fraction (S1) and the remainder (S2). In S1 a substance giving a 2 banded fluorescent spectrum above 400 mμ was detected in minute amounts in 6 of 8 attempts. It cannot be stated whether this was naturally produced or an external contamination acquired during lifelong accumulation. A second fluorescent substance which was found in large amounts and which gave a broad-banded fluorescent spectrum below 400 mμ was shown to be a normal cyclization product of squalene which is present in abundant quantities. After nine months application to skin pockets of mice, S1 produced no tumors and S2 one papilloma.

A colorimetric method has been developed for the determination of squalene and this substance has been shown to be present in several sebum-like substances of human origin. It is suggested that aberrant cyclization of squalene in skin followed by aromatization could lead to the formation of a carcinogen. It was determined that rat sebum differs from human sebum and lanolin. Therefore, the composition of sebum may condition species response to painting with carcinogen. It is suggested that triterpenoids may play some role in carcinogenesis and anticarcinogenesis.

MECHANISMS OF METASTASIS. I. Zeidman (Introduced by Dale Rex Coman). (Department of Pathology, University of Pennsylvania, School of Medicine, Philadelphia 4, Pa.)

Previous investigations in this laboratory on invasiveness of cancer were continued by a study of the conditions that determine the number of metastases from a transplanted tumor. The factors studied were size and number of transplanted tumors, and their duration. The transplantable fibrosarcoma, T-241, was inoculated into one or both flanks of C 57 black mice. Pairs of mice bearing single tumors and pairs bearing double tumors were sacrificed on the same day at intervals from 9 to 26 days after tumor inoculation. The tumor volumes were measured by fluid displacement, and the lung metastases were counted. There was no significant relationship between number of lung metastases and primary tumor volume. The number of lung metastases produced by two tumors in the same host was not different from the number of metastases produced by a single tumor. The correlation between number of metastases and duration of the primary tumor was significantly positive. In cancer of man, where hosts and tumors are variables, there is no apparent correlation between the size of the primary tumor and the number of metastases. Surprisingly, in the above experiments, where host and tumor variables were largely controlled, the same lack of correlation between size of primary tumor and number of metastases was apparent.

RESPONSE OF THE CENTRAL NERVOUS SYSTEM OF THE CHICKEN TO METHYLCHOLANTHRENE: FAILURE TO INDUCE TUMORS AFTER FOUR YEARS OF STIMULATION. William O. Russell and George S. Loquvam (by invitation). (The M. D. Anderson Hospital for Cancer Research, Houston, Texas)

Previous experiments indicating that tumors of nervous tissue origin could be regularly induced in the brains of rats and mice with methylcholanthrene and that in the rat a diet deficient in certain B vitamins influenced the induction period, suggested further study of the problem in fowls.

Pedigreed white leghorn chickens were selected for the experiment and pellets of 30 per cent methylcholanthrene fused with chemically pure cholesterol were implanted in the right cerebral hemisphere. A thiamine deficient diet was given at intervals to one group, followed by the injection of thiamine hydrochloride intramuscularly and a period of normal diet. It was only possible to give three periods of thiamine deficiency because the fowls were moved to another laboratory where it was not possible to continue the dietary phase of the problem. The periods of deficiency were given in a four month period. None of the chickens developed tumors. Six chickens were sacrificed 4 years and 8 months after implantation of the carcinogen; 10 chickens survived 3 years and 15 chickens survived 2 years. These results indicate that the central nervous system of the chicken is resistant to the carcinogenic stimulation of methylcholantrene in concentrations that regularly produce neoplasms of the brain in rats and mice.

AN EXPERIMENTAL STUDY OF THE EFFECTS OF MALIGNANCY AND DIABETES ON EACH OTHER. Alan W. Carrie (by invitation) and Arthur W. Ham. (Department of Anatomy, University of Toronto, Toronto, Canada.)

Sarcoma 37 was transplanted into rats made diabetic with alloxan and into normal rats. Tumors were observed to grow just as rapidly in the diabetic rats as in the non-diabetic ones. The blood sugar level was followed for 2 months in 12 diabetic rats with growing tumors and in 13 normal rats with comparable diabetes. No significant difference between blood sugar levels in the two groups was observed. A growing tumor in a diabetic animal was not found to affect the rate at which its blood sugar level fell on starvation.

The fact that tumors were found to grow just as quickly in diabetic rats as in non-diabetic controls suggests that the metabolic processes of the malignant cell are not dependent on insulin. Since tumor cells obtain a large proportion of their energy requirements from fermentation, these findings suggest further that none of the reactions concerned in glycolysis are dependent on insulin Nevertheless, even though tumors can glycolyze sugar in the absence of insulin this experiment indicates that the demands of a tumor on an organism for sugar are not sufficient to substantially lower its blood sugar level and hence that the diminution of glycosuria noted in diabetics (before the advent of insulin) when they

developed malignant tumors is probably not to be explained by the tumors, as a result of their glycolytic activities, having substantially reduced the blood sugar levels of the individuals concerned.

THE INFLUENCE OF CERTAIN SULFONAMIDE DRUGS ON CANCER SUSCEPTIBILITY AND REPRODUCTION IN MICE.*

FRANK H. J. FIGGE,† and GERALDINE F. WOLFE, ROSALIE YERKES FIGGE (by invitation). (University of Maryland School of Medicine, Baltimore 1, Md.)

The influence of sulfanilamide, sulfathiazole, and sulfapyridine on the cancer susceptibility of mice was tested over a five year period. A total of 4353 mice of various strains (C3H, C57, A) were treated throughout life with the various sulfonamide drugs. In some cases, the experimental period involved 8 generations of mice receiving continuous treatment. No significant effect of sulfonamide treatment on cancer susceptibility was observed, but it was found that sulfanilamide in a concentration of 3 grams per liter in the drinking water inhibited reproduction. The inhibitory influence of sulfapyridine on reproduction was not so marked and did not extinguish the lines until the third- or fourth-treated generation. Sulfathiazole had little or no effect on reproduction and the mice treated continuously with this drug were followed through 8 generations.

An attempt was made to investigate the nature of this inhibition of reproduction by sulfanilamide. The reproduction inhibiting potentialities of sulfonamide drugs appeared to parallel the tendency of the various drugs to produce Heinz bodies in the erythrocytes of mice; but this parallelism may be coincidental. The inhibition of reproduction by sulfanilamide could not be counteracted by administration of large supplements of various vitamins to the diet. Feeding thyroid also failed to produce any appreciable change in the reproductive ability of sulfanilamide-treated mice.

FURTHER STUDIES ON HYPERVOLEMIA AND ASSOCIATED CHANGES IN MICE BEARING A TRANSPLANTED GRANULOSA-CELL TUMOR.

JAMES T. WOLSTENHOLME (Introduced by W. U. GARDNER). (Department of Anatomy, Yale University School of Medicine, New Haven, Conn.)

Mice bearing a transplanted granulosa cell tumor have hypervolemia and dilatation of the sinusoids of the liver, spleen and adrenal glands. (Furth, Boon, and Sobel, 1945.)

Four groups of experiments were set up:

Parabiotic series.—Animals of the C57 strain were castrated at 60 to 90 days of age and small fragments of a granulosa cell tumor were transplanted subcutaneously. When the tumor transplant was approximately 3 to 4 mm. in diameter, the animal was parabiosed to a littermate. When the tumor attained 1 mm. in diameter or more, the tumor bearing animal had hypervolemia and liver changes; no changes occurred in the non-tumor parabion twin.

Splenectomy series.—Small fragments of a granulosa cell tumor were transplanted subcutaneously into castrated animals of the C57 strain. When the tumor transplant was 3 to 8 mm. in diameter the animal was splenectomized. These animals developed hypervolemia and liver changes.

Tumor removal experiment.—Eight mature C57 animals bearing granulosa cell tumors of different sizes and having hypervolemia and liver changes had the tumors removed. When sacrificed 5 days later there was no hypervolemia or liver change present.

Tumor extract experiment.—Eighty-three grams of fresh granulosa cell tissue obtained from C57 mice were extracted with acetone. The acetone extract was evaporated and redissolved in sesame oil. The acetone insoluble fraction was dried, powdered and suspended in water. Daily injections of the acetone extract into C57 animals and daily injections of the aqueous suspension into C57 animals for an 8 week period failed to produce the hypervolemia or associated changes that were produced by the tumor.

EFFECTS OF A NITROGEN MUSTARD ON THE CICHLID FISH, *TILAPIA MACROCEPHALA* (BLEEKER).

SOPHIE JAKOWSKA (by invitation), and R. F. NIGRELLI. (College of Mount St. Vincent and New York Zoological Society, New York, N.Y.)

Young mature fish, 4 to 6 cm. in standard length, from a common stock were placed in tanks containing 4 liters of homotypic conditioned water in which was dissolved enough methyl-bis (beta-chloroethyl) amine hydrochloride to make concentrations of 0.0017 and 0.002 per cent. The tanks, each containing 4 fish, were continuously aerated and kept at room temperature, The fish showed hyperemic gills at the end of 24 hours. At the end of 48 hours, desquamation of the epithelium of the gills, oral and branchial cavities was evident and there also occurred a general nephrosis with glomerular and tubular degeneration. In all fish there was an invasion by macrophages and eosinophiles in the tissues adjacent to the branchial region in response to a parasitic infection by the ciliate, *Ichthyophthirius multifilis.* In treated fish, the parasites were absent but the massed macrophages and eosinophiles were still present, indicating that the nitrogen mustard, in the concentration and time used, had no apparent effect on these cells. With the exception of the testis, the other internal organs including the blood, showed no striking histological changes. Feulgen-stained smears of the testis showed that divisions were arrested, since only cells in the resting stage and in prophase were observed, whereas in untreated fish all stages of mitosis and meiosis were encountered. Fish exposed to 0.01 per cent concentration for 1 hour did not show any significant tissue changes, indicating that the effects of the nitrogen mustard, as employed under these conditions, were manifest only after comparatively longer exposures.

* Aided by grants from the Anna Fuller Fund and the Donner Foundation.

† Visiting Professor of Anatomy, University of Pennsylvania, School of Medicine, Philadelphia, Pennsylvania.

THE QUESTION OF A THYROID GROWTH CENTER.

JOHN H. VAN DYKE. (Indiana University School of Medicine, Bloomington and Indianapolis, Ind.)

During development in mammals an ultimalbranchial body, derived from the hind end of the embryonic pharynx, comes to lie within the center of each lateral lobe of the thyroid where apparently it is frequently induced to form functional thyroid parenchyma.

In rats after birth ultimobranchial tissue, normally indistinguishable from thyroid tissue, may be similarly modified by feeding vitamin A deficient diet, administration of estrogen or methylcholanthrene. Following early hyperplasia, this tissue usually transforms, through metaplasia, into multiple cysts lined by stratified squamous epithelium whereas adjacent peripheral thyroid follicles undergo general atrophy. Normal old age rats frequently exhibit this condition; not infrequently associated with cystadenomata.

In rats, the site of maximal mitoses appears to be in the center of each thyroid lobe, both in normal growing animals and during experimental hyperplasias. When thyroids containing ultimobranchial cysts are stimulated spontaneously (sheep) or, under optimum conditions, in rats by cold or hypophyseal extract, mitotic division of thyroid parenchyma does not ensue. Apparently, however, there is a compensatory proliferation of masses of relatively indifferent cells from the bases of ultimobranchial cysts which transform, upon demand, into typical thyroid tissue in a manner to be described.

Evidence suggests that ultimobranchial tissue is plastic, normally indistinguishable from thyroid tissue, and may function after birth as a thyroid growth center or, being labile, may undergo cyclic phenomena depending upon factors altering thyroid activity Conditions augmenting thyroid hyperactivity in mammals (including man), particularly those with ultimobranchial lesions, may predispose to certain compensatory neoplasms—especially during senility.

RESULTS OF THE BIOLOGICAL TEST FOR MALIGNANCY.

HOWARD H. BEARD. (Cancer Clinic, Holy Cross Hospital, Chicago, Ill.)

Four hundred urines from malignant patients (biopsy in 36 per cent) were run by the biological test and 94 per cent of them gave positive results. These were from 55 different sites (breast, 50; uterus, 39; stomach, 33; lungs, 27; cervix, 26; colon, 16; leukemia, 20; etc.). In 221 nonmalignant urines there were 87 per cent negative tests. In 183 miscellaneous urines there were 62 or 34 per cent positive and of these 11 were from pregnancy and postpartum urines. In 67 normal urines 97 per cent were negative. These results agree closely with those of Roffo (1), Sakai (2), and Aron (3), and further confirm the validity of the trophoblastic thesis of malignancy. The following applications of the biological test were suggested: (a) in supplementing clinical and pathological diagnosis of malignancy, (b) in testing for the presence or absence of metastases after treatment, (c) in controlling chymotrypsin therapy, (d) in distinguishing between the presence of a benign and malignant tumor in the patient, and (e) in detecting the presence of early malignancy in a given individual. A positive test in a malignant patient will become negative after 2 or 3 weeks of chymotrypsin therapy (total from 100 to 300 mgs.) in those patients who survive. In those that don't the test may remain positive in spite of enzyme therapy. Another investigator has discovered the presence of a chymotrypsin inhibitor only in the sera of pregnant women and malignant patients. The antichymotrypsin and biological tests agreed closely in 43 out of 46 cases.

1. Roffo, A. H. Bol. Inst. Med. Exper., No. 65, 419, 1944.
2. Sakai, S., Igaku Kenyu, **13,** 1, 1939.
3. Aron, M., Presse med., **43,** 1044, 1935.

CARCINOGENIC ACTION OF CERTAIN CATALYTICALLY CRACKED OILS WITH HIGH BOILING POINTS.

KANEMATSU SUGIURA, WILLIAM E. SMITH, AND DOUGLAS SUNDERLAND (by invitation). (Sloan-Kettering Institute for Cancer Research, New York, N.Y.)

In collaboration with a petroleum company* 200 samples of oils, waxes, and tars were tested for carcinogenicity by painting upon the skin of animals. Samples of petroleum submitted to catalytic cracking at high temperatures exhibited marked carcinogenicity.

Mice painted with high boiling catalytically cracked oils showed, in certain cases, papillomas as early as 29 days and squamous cell carcinomas as early as 70 days. With continued painting, papillomas developed in nearly every animal and in a majority underwent malignant change. The same material elicited papillomas in all of 6 rhesus monkeys after 3 years of painting, but no tumors were obtained in guinea pigs and only 1 in 59 rats living over 1 year. Papillomas arose in all and cancers in 3 of 26 rabbits.

Certain samples of these catalytically cracked oils showed a carcinogenic potency nearly comparable to 0.3 per cent solutions of methylcholanthrene in acetone. The carcinogenic material was in the fractions distilling over above 700° F. The carcinogenic material can be concentrated in the aromatic fractions after discarding heptane-insoluble matter and adsorbing on silica gel columns followed by elution with cumene or acetone.

* These studies were carried out under a grant from the Standard Oil Company (New Jersey) and certain affiliates in cooperation with the Medical Department of that Company. All samples were prepared and supplied by the Standard Oil Development Company.

American Association for Cancer Research, Inc.

40th Annual Meeting

Hotel Fort Shelby, Detroit, Michigan

April 16 and 17, 1949

Proceedings of Business Sessions

Minutes of the Meeting of the Board of Directors Held April 15, 1949

The meeting was held in the Commerce Room of the Hotel Fort Shelby in Detroit, Michigan, and was called to order at 7:05 P.M. by the Chairman of the Board. Present were Board members J. C. Aub, J. J. Bittner, A. M. Brues, E. V. Cowdry, E. A. Doisy, J. Furth, C. W. Hooker, Charles Huggins, and H. C. Taylor, Jr., and Dr. Theodore P. Eberhard, Business Manager of *CANCER RESEARCH*.

The minutes of the last meeting were read and approved.

REPORTS OF OFFICERS

The President reported that the chief interest of his office had been in *CANCER RESEARCH*, pursuant to the agreement at the last meeting that the Association assume responsibility for the journal. At that time *CANCER RESEARCH* was several months in arrears of publication and its editor wished to be relieved of his duties. Acting with the approval of Mr. W. H. Donner of Donner Foundation, Cancer Research, Inc., a holding company consisting by definition of the three officers of the Association, was incorporated in Wilmington, Delaware on June 17, 1948. On September 19, 1948 a meeting of the Board of Managers of *CANCER RESEARCH*, appointed by the Board of Directors of the Association, was held in Washington. Present were Drs. Balduin Lucké, P. E. Steiner, E. W. Shrigley, Albert Tannenbaum, Theodore P. Eberhard, and Charles Huggins. After considering estimates of several publishing houses, the Board of Managers agreed upon the University of Chicago Press as Printer and Dr. Paul E. Steiner as Editor-in-Chief. Both Doctor Steiner and the Press accepted their appointments. The Board of Managers recommended that the "abstracts section" in *CANCER RESEARCH* be discontinued. Applications for grants in aid for 1949 were then made to the Jane Coffin Childs Fund for $5,500; the Anna Fuller Fund for $2,000; and the Pardee Foundation for $1,000. These organizations generously agreed to these subsidies, thus meeting the conditions set by Mr. W. H. Donner for his subsidy of $5,000 for 1949. The annual cost of publication is roughly $25,000. On October 25, 1948 an agreement was reached between the University of Chicago Press and Cancer Research, Inc. for publication until January 1, 1951, and on a year to year basis thereafter, with the understanding that after January 1, 1951 either party may terminate the arrangement on ninety days notice. Due to the effective work of Dr. Lucké and his staff, ably aided by Doctor Eberhard, the Ann Arbor Press has completed publication of Volume 8, with the exception of the December issue which is now ready for final printing. On November 22, 1948 the Ann Arbor Press agreed to terminate its contract with Cancer Research, Inc. on completion of Volume 8. On January 25, 1949 the January 1949 number of *CANCER RESEARCH* appeared under the aegis of Dr. P. E. Steiner's new editorial board and the new printer. Since then issues have appeared regularly and on schedule. The Press deals with subscriptions to back volumes and to current subscriptions of non-members. The Press is also investigating the possibility of obtaining advertising, but they consider advertising inadvisable until subscriptions total at least 1500. The present circulation is approximately 1300. When the circulation reaches 2500, advertising can provide a good source of support. On March 25, 1949 Editor-in-Chief Steiner notified Cancer Research, Inc. that he will not be available as Editor upon completion of Volume 9. On April 1, 1949 the monies of Cancer Research, Inc. on deposit with the Fidelity-Philadelphia Trust Company totalled $19,620.-05. We are now publishing a journal of 64 pages that is appearing promptly. Cancer Research, Inc. and the Board of Managers of *CANCER RESEARCH* believe that the cost of the journal to subscribers and to members of the Association is too low, and they recommend an increase to both classes of subscribers. The President reiterated his conviction that the publication of *CANCER RESEARCH* is a vital function of the Association. He emphasized that Cancer Research, Inc. is indebted particularly to Balduin Lucké, Paul E. Steiner, and Theodore P. Eberhard for their heroic efforts to transfuse and transform *CANCER RESEARCH;* that we are singularly in the debt of Mr. William H. Donner, the Childs and Fuller Funds, and the Pardee Foundation, without whose assistance nothing could have been attempted; that Cancer Research, Inc. is most grateful to the other members of the Association for their patience and support in this trying period.

The President concluded his report by restating his

conviction that the Association is a non-political group, organized to disseminate information, and to provide annual opportunity for meeting for comfort, encouragement, and exchange of ideas in a convivial atmosphere.

It was voted that the report of the President be accepted with commendation.

The President, in the absence of Dr. Balduin Lucké, then presented the report of the Board of Managers of *CANCER RESEARCH*. The report recommended the abolition of the Board of Managers and its replacement by an Executive Council to be composed of the immediate Past President, President, Vice-President, and Secretary-Treasurer of the Association, and the Editor-in-Chief of *CANCER RESEARCH*. It was recommended that the Executive Council handle administrative problems of the journal and problems of the Association needing prompt action in the interval between annual meetings of the Association and its Board of Directors; that editorial policies of the journal be governed by the Editorial Board of the Journal. After brief discussion, it was voted that the recommendations of the Board of Managers of *CANCER RESEARCH* be adopted. The report of the Board of Managers also urged increasing annual dues of active members to ten dollars, eight dollars of which would go to *CANCER RESEARCH* and two dollars of which would be applied to the expenses of the Association. It was voted to accept the recommendation and to refer it to the members of the Association at their meeting.

It was then voted that the Secretary appropriately thank the Editors of *CANCER RESEARCH* on behalf of the Association for their effective labors in bringing out *CANCER RESEARCH*.

The Secretary reported that Dr. Vasant Ramji Khanolkar of Bombay, India had accepted the Honorary Membership to which he was elected at the last annual meeting.

The Secretary reported that comparatively little objection to the increase in annual dues from three to seven dollars had come to his attention. One member proposed to the Secretary that two classes of membership be created, full membership corresponding to the pres-

TREASURER'S REPORT

March 31, 1949

Journal Fund				
On deposit (Union and New Haven Trust Co.) 3–1–48			$ 81.00	
Interest, 3/1/48 to 3/31/49			.80	
				$ 81.80
Savings Account				
On deposit (Greenwich Savings Bank) 3–1–48			654.34	
Interest, 3/1/48 to 3/31/49			9.84	
				664.18
Operating Funds				
On deposit (Union and New Haven Trust Co.) 3–1–48			2,775.40	
Receipts, Dues			3,856.20	
Subscriptions to back volumes *Cancer Research*			45.00	
				6,676.60
Disbursements				
Cancer Research, contribution, 1948		$ 602.00		
Cancer Research, subscription 1948, one member		5.00		
University of Chicago Press				
464 subscriptions @ $6.00	$2,784.00			
4 supplements to paid subscriptions	4.00			
2 subscriptions @ $6.00, less $5.00 credit	7.00			
Overpayment	12.00	2,807.00		
Traveling expenses, Board of Managers, *Cancer Research*		136.21		
Annual Meeting, 1948				
Printing (Programs, etc.)	160.75			
Slide projectors, services	146.30			
Telephone, shipping charges	6.17	313.22		
Secretary's office				
Secretarial assistance	250.00			
Printing, office supplies	180.14			
Postage, stamps	63.91			
Addressograph plates (605), 3 drawers	51.11	545.16		
Refunds on overpaid dues		5.00		
Bank charge, Canadian check		.27		
Reprints, minutes of Directors' meeting Oct. 13, 1946		.55		
				4,455.09
BALANCE, March 31, 1949				$2,221.51

I hereby certify that the accounts and vouchers in the American Association for Cancer Research, Inc., for the above recorded period, have been examined by me, and that the above are true statements of its financial operations and of its financial conditions as of March 31, 1949.

THEODORE P. EBERHARD
Auditor for the Directors

ent active membership and associate membership not carrying with it a subscription to *CANCER RESEARCH* and costing three dollars annually. Another member raised the question whether a husband and wife, both members of the Association, should both pay full dues and both receive *CANCER RESEARCH*. To date 470 members have paid dues for 1949. The request of several members that a divided statement of dues be rendered, one that will allow these members to pay what they consider to be their dues and their laboratory to pay for the journal, was mentioned. The Secretary raised the question whether he has the authority to meet these requests. After brief discussion the following actions were taken. With respect to the establishment of an associate membership, it was voted that the By-Laws be affirmed. It was agreed with respect to full payment of dues by husband and wife that no special arrangements should be made at present. With reference to preparing divided statements, the Board voted "that the Secretary be instructed to issue a lump-sum statement of dues," the sense of the group being that dues are a set sum in return for which the member receives various rights and privileges, including *CANCER RESEARCH*, and that reimbursement is the problem of the member.

The Treasurer's annual report was then read and accepted, pending approval by an auditor. Dr. Theodore P. Eberhard was appointed auditor.

REPORTS OF STANDING COMMITTEES

Program Committee.—Chairman J. C. Aub reported that the number of abstracts submitted greatly exceeded that of any year in the past, and that his Committee attempted to place as many different investigators on the program as possible. Even with a two-day meeting with three sessions running concurrently, one-third of the papers submitted had to be read by title. In the discussion it was agreed that papers read by title are to be a permanent portion of the program. With respect to the next meeting, the Board voted to hold a three-day meeting to be held more or less in conjunction with the meeting of the Federation of American Societies for Experimental Biology, the precise time to be determined by the Program Committee.

Nominating Committee.—Chairman Hooker reported that his Committee had proposed for new members of the Board of Directors Drs. J. C. Aub, E. V. Cowdry, E. A. Doisy, W. U. Gardner, H. P. Rusch, M. B. Shimkin, H. C. Taylor, Jr., and Albert Tannenbaum. These names were listed on the proxy ballots sent to the members of the Association by the Secretary. Count of the ballots revealed that Doctors Aub, Cowdry, Doisy, and Gardner received the largest number of votes. The Board voted, "That the Secretary cast one ballot for the nominees chosen by the members." The new Directors were then declared elected.

Membership Committee.—Chairman Jacob Furth reported that the Association now has 604 active members, 4 emeritus members, 3 contributing members, and 8 honorary members. The resignations of the following members were submitted and accepted:

C. J. Christy
Arthur F. Coca
Charles A. Elsberg
William R. Franks
J. E. Gendreau
A. W. Hengerer
Martha Elizabeth Howe
Richard Lewisohn
Catharine Macfarlane
William C. McCarty
Angelo H. Roffo
Isabel M. Scharnagel
Fritz Schlenk
Harold A. Solomon
William H. Wehr
Florence R. White
Thomas N. White

The requests of Dr. C. H. Bunting, Dr. B. J. Clawson, Dr. Thomas S. Cullen, and Dr. E. E. Tyzzer for transfer to Emeritus Membership were submitted and approved.

The deaths of the following members were announced with expressions of profound regret.

Montrose T. Burrows
Mortimer Cohen
Joseph McFarland
A. A. Thibaudeau

The nominations for active membership were then presented. The Committee recommended the election of 66 nominees. They were:

Arneson, Axel Norman, M.D., Washington University School of Medicine, St. Louis 8, Mo.

Berenblum, Isaac, M.D., National Cancer Institute, Bethesda 14, Md.

Bodansky, Oscar, Ph.D., M.D., Memorial Hospital, 444 East 68th Street, New York 21, N.Y.

de Bruyn, Willemina M., Ph.D., Johns Hopkins Hospital, Baltimore 5, Md.

Burchenal, Joseph H., M.D., Sloan-Kettering Institute for Cancer Research, 444 East 68th Street, New York 21, New York.

Cambel, Perihan, M.D., Washington University School of Medicine, St. Louis 10, Mo.

Chalkley, Harold W., Ph.D., National Cancer Institute, Bethesda 14, Md.

Clark, R. Lee, Jr., M.D., M.D. Anderson Hospital, 2310 Baldwin Street, Houston 6, Texas.

Cook, Elton S., Ph.D., Institutum Divi Thomae, 1842 Madison Road, Cincinnati 6, Ohio.

Copeland, D. H., B.S., Alabama Polytechnic Institute, Auburn, Alabama.

Davidsohn, Israel, M.D., Mt. Sinai Hospital, 2750 West 15th Place, Chicago 8, Ill.

Elman, Robert, M.D., Washington University School of Medicine, St. Louis 10, Mo.

Everett, Mark R., University of Oklahoma School of Medicine, Oklahoma City 4, Okla.

Fishman, William H., Ph.D., Tufts College Medical School, Boston 11, Mass.

Frantz, Ivan DeRay, M.D., Massachusetts General Hospital, Boston 14, Mass.

Gorbman, Aubrey, Ph.D., Barnard College, Columbia University, New York 27, N.Y.

Gorham, L. Whittington, M.D., Albany Medical College, Albany 1, N.Y.

Greenberg, David M., Ph.D., University of California Medical School, Berkeley 4, Calif.

GRIER, ROBERT S., M.D., Massachusetts General Hospital, Boston 14, Mass.
GRIFFIN, AMOS CLARK, PH.D., Stanford University, Stanford, Calif.
HARNLY, MORRIS H., PH.D., New York University, New York 3, N.Y.
HEIDELBERGER, CHARLES, PH.D., University of Wisconsin, Madison 6, Wis.
HOPPS, HOWARD C., M.D., University of Oklahoma School of Medicine, Oklahoma City, Okla.
HUGULEY, CHARLES M., JR., M.D., Emory University School of Medicine, Emory University, Ga.
JONES, RALPH, JR., M.D., University of Pennsylvania Hospital, Philadelphia 4, Pa.
KENNEDY, BYRL J., M.D., Massachusetts General Hospital, Boston 14, Mass.
KING, JOSEPH T., PH.D , M.D., University of Minnesota, Minneapolis 14, Minn.
KREKE, CORNELIUS W., PH.D., Institutum Divi Thomae, 1842 Madison Road, Cincinnati 6, Ohio.
LANSING, ALBERT I., PH.D., Washington University School of Medicine, St. Louis 10, Mo.
LAWRENCE, EDWIN A., M.D., University of Utah College of Medicine, Salt Lake City 5, Utah.
LEATHEM, JAMES H., PH.D., Rutgers University, New Brunswick, N.J.
LENTA, SISTER M. PETRA, M. S., College of St. Scholastica, Duluth 2, Minn.
LEWIS, GEORGE T., PH.D., Emory University School of Medicine, Emory University, Ga.
LUCK, J. MURRAY, PH.D., Stanford University, Stanford, Calif.
LUSHBAUGH, CLARENCE C., PH.D., M.D., Los Alamos Scientific Laboratory, Los Alamos, New Mexico.
MAUN, MARK E., M.D., St. Mary's Hospital, 1420 St. Antoine, Detroit 26, Mich.
MELLORS, ROBERT C., PH.D., M.D., Sloan-Kettering Institute for Cancer Research, 444 East 68th Street, New York 21, N.Y.
MOORE, ALICE E., M.D., Sloan-Kettering Institute for Cancer Research, 444 East 68th Street, New York 21, N.Y.
MULLIGAN, RICHARD M., M.D., University of Colorado School of Medicine, Denver 7, Col.
NEWTON, BERNE L., M.D., Baylor University College of Medicine, Houston 1, Texas.
NOVIKOFF, ALEX B., PH.D., University of Vermont College of Medicine, Burlington, Vt.
NUTINI, LEO G., M.D., Institutum Divi Thomae, 1842 Madison Road, Cincinnati 6, Ohio.
ODELL, LESTER D., M.D., University of Chicago, Chicago 37, Ill.
OPPENHEIM, ABRAHAM ,M.D., 30 East 60th Street, New York 22, N.Y.
PALETTA, FRANCIS X., M.D., 634 N. Grand, St. Louis 3, Mo.
PETERMANN, MARY LOCKE, PH.D., Sloan-Kettering Institute for Cancer Research, 444 East 68th Street, New York 21, N.Y.
PINKERTON, HENRY, M.D., St. Louis University School of Medicine, St. Louis 4, Mo.
RILEY, VERNON T., National Cancer Institute, Bethesda 14, Md.
ROSENTHAL, THEODORE B., PH.D., Washington University School of Medicine, St. Louis 10, Mo.
RUGH, ROBERTS, PH.D., Columbia University, New York 32, N.Y.
SALMON, WILLIAM D., M.A., Alabama Polytechnic Institute, Auburn, Ala.
SCOTT, WILLIAM W., PH.D., M.D., Johns Hopkins Hospital, Baltimore 5, Md.
SHACTER, BERNARD, PH.D., Laguna Honda Home, San Francisco 16, Calif.
SHAY, HARRY, M.D., Temple University School of Medicine, Philadelphia, Pa.
SHETLAR, MARVIN R., PH.D., University of Oklahoma School of Medicine, Oklahoma City 4, Okla.
SKAPIER, JOSEPH, M.D., PH.D., 330 West 72nd Street, New York 23, N.Y.
STERN, KURT G., PH.D., Polytechnic Institute of Brooklyn, Brooklyn 2, N.Y.
STEVENS, CHARLES D., PH.D., University of Cincinnati College of Medicine, Cincinnati 19, Ohio.
SUMMERSON, WILLIAM H., PH.D., Army Chemical Center, Md.
SYMEONIDIS, ALEXANDER, M.D., PH.D., National Cancer Institute, Bethesda 14, Md.
THURINGER, JOSEPH, M.D., University of Oklahoma School of Medicine, Oklahoma City 4, Okla.
WARD, GRANT E., M.D., 214 Medical Arts Building, Baltimore 1, Md.
WARTMEN, WILLIAM B., M.D., Northwestern University Medical School, Chicago 11, Ill.
WIGHT, KENT M., PH.D., National Cancer Institute, Bethesda 14, Md.
WILSON, J. WALTER, PH.D., Brown University, Providence 12, R.I.
ZEIDMAN, IRVING, M.D., University of Pennsylvania School of Medicine, Philadelphia 4, Pa.

Committee on Cancer Research, Its Organization and Support.—The report, submitted by Chairman Shields Warren, has been filed in the office of the Secretary. By order of the Board an abstract of the report is given here:

> The most striking development since the last report has been the establishment of a program for cancer research in the field of atomic energy. The initial appropriation made by Congress to the Atomic Energy Commission had $4,000,000 earmarked for cancer research. The program has consisted of development of facilities for clinical investigation in conjunction with the national laboratories; provision of funds for the Atomic Casualty Committee of the National Research Council for study of the carcinogenic potentiality of a single dose of radiation in the human material at Nagasaki and Hiroshima; provision of funds for research in certain fields having to do with the utilization of radioactive isotopes for research in therapy; advancement of funds to the Office of Naval Research to permit continuation of cancer research projects threatened by the insufficient funds of the Office of

Naval Research; making available free in April, 1948 the isotopes P^{32}, I^{131}, and Na^{24} for cancer research and treatment; making available free in February 1949 all other isotopes for cancer research. Only shipping and handling charges must be paid by the investigator. For this purpose the Atomic Energy Commission has set aside $450,000. The Isotope Distribution Committee of the Atomic Energy Commission and its Subcommittee on Human Allocations are doing effective work in making isotopes widely available and in insuring the safety of those who may come in contact with them.

A second important development is the initiation of a program of construction of research facilities by the U.S. Public Health Service. The program is the outgrowth of recognition by the Public Health Service that universities have insufficient funds for new construction, that present facilities are overcrowded, and that effective use of the brains and money available for cancer research is contingent upon space to work. The first step was authorized by the Eightieth Congress, and that policy is apparently to be continued by the present Congress.

A significant trend toward increased emphasis on chemotherapy is pointed out.

It is noted that most of the privately endowed foundations are now placing little emphasis on cancer, recognizing the sums provided by other sources. The foundations specifically for cancer, such as the Anna Fuller Fund and the Childs Fund, continue their effective support. The M. D. Anderson Foundation is gradually developing a potentially effective cancer center in Houston, and we expect this Foundation to be a source of future impetus to cancer research.

The report records the establishment of the new journal *CANCER*, and expresses satisfaction with the assumption of responsibility for *CANCER RESEARCH* by the Association. The publication of the "Index to Literature of Experimental Cancer Research, 1900 to 1935" by the Donner Foundation is regarded an important service to investigators.

The great number of meetings on various aspects of the cancer problem held since the war is mentioned. The Committee recognized the value of these meetings, but suggests that there is the possibility of too many meetings retarding rather than expediting research. The growing international cooperation in cancer research, however, is enthusiastically endorsed.

The reconstruction of the Roscoe B. Jackson Memorial Laboratory is recorded as being of great significance.

As to the future, the report considers it obvious that increased clinical research on patients is needed. The recent advancements in the laboratory need to be checked by pilot clinical studies. A great need is research wards whose cost will not be met directly by the hospitals. Significant exploration in this field is being made by the U.S. Public Health Service. The fact that project research of a short-term character is not fully effective is again urged. The advantages of the institutional type of grant as developed by the American Cancer Society are mentioned. The occasional tendency within the recipient institution to break up the grant into a series of project grants is decried.

The report concludes with a restatement of the increased costs of research and of the fact that much current research is a painstaking survey of fields already roughly mapped out. Costly apparatus and teams composed of men familiar with a number of diverse scientific fields are needed.

REPORTS OF SPECIAL COMMITTEES

Committee on Local Sections of the Association.—Chairman E. V. Cowdry reviewed the survey made by his Committee and their recommendations, and presented from his Committee the following resolution to serve as a guide for an addition to the By-Laws of the Association:

> "The American Association for Cancer Research may have local chapters in such areas which have sufficient numbers of individuals with scientific interest in cancer research. Application for establishment of the chapters shall be made by the local group of no less than 10 members of the Association, and shall be considered by the Board of Directors; if the Board agrees that the geographic area and other conditions are acceptable, the authorization for the chapter shall be made by the vote of the members at their annual meeting. Additional members of the chapter do not necessarily have to be members of the Association, if they meet the same general qualifications as members of the Association, with the exception of the publication requirement. Meetings of the chapters shall be held at least twice a year, and reports of such meetings shall be made to the Secretary of the Association."

The Board voted to accept the report and to adopt the resolution.

REPORTS OF REPRESENTATIVES OF THE CORPORATION

Dr. E. V. Cowdry, delegate to the International Cancer Research Congress, reported that a clarification of the Congress is expected at the meeting in Paris in the summer.

It was voted "That Drs. E. V. Cowdry and W. U. Gardner be recommended by the Association as official delegates of the United States to the International Cancer Research Commission, the recommendation to be transmitted by the Secretary to Dr. Ignacio Y. Milan in Mexico by order of the President of the Association."

UNFINISHED BUSINESS

After brief discussion it was voted not to affiliate with the Union of Biological Societies at the present time.

It was reported that a history of the Association is now in preparation.

NEW BUSINESS

In view of the inconstancy in the date of the annual meeting from year to year and because of the increased financial operations of the Association, it was voted, "That the fiscal year of the Association henceforth cover the period January 1 through December 31." The Board also voted to approve the briefest possible summary of activities in the published version of the Treasurer's report.

Authorization was given the Secretary-Treasurer to make such alterations in the composition of the annual statements of dues as are necessary.

Dr. E. V. Cowdry reported that plans have been made for the Fifth International Cancer Research Congress to be held in Paris in 1950, probably in July. Invitations have already been sent, and the President will be Dr. A. Lacassagne. It has been agreed to request the International Congress to send every member of the Association an invitation to present a paper.

It was pointed out that in the voting of members for new members of the Board of Directors during the last two years the largest vote has been cast for the first four names on the ballot. It was also pointed out that it has become customary for the Nominating Committee to renominate the retiring members of the Board, that any new Committee and any retiring Director is thrown into embarrassing circumstances if an unintentionally self-perpetuating Board is to be made impossible. After discussion it was voted, "That the order of names on the ballot for Directors be determined by lottery, and that this fact be stated on the ballot." It was further voted that "Beginning in 1951 no member of the Board of Directors can succeed himself in office for a period of one year."

Nominations for officers of the Association for the coming year were then made; for President, Joesph C. Aub; for Vice-President, Jacob Furth, for Secretary-Treasurer, Charles W. Hooker.

It was voted to hold the next meeting of the Board at noon on April 17, 1949.

The meeting was adjourned at 10:15 P.M.

CHARLES HUGGINS
Chairman, Board of Directors

CHARLES W. HOOKER
Secretary

MINUTES OF THE MEETING OF THE MEMBERS HELD APRIL 16, 1949

The meeting was held in the Coral Room of the Hotel Fort Shelby and was called to order at 1:48 P.M.

Reading of the minutes of the last meeting was omitted by vote of the members.

The Treasurer's report and the report of the Auditor were read and accepted.

Dr. E. V. Cowdry, Chairman of the Committee on Local Sections, reported the work of his committee and submitted the Resolution regarding the establishment of Sections of the Association recorded in the minutes of the meeting of the Board of Directors on April 15, 1949. It was voted to adopt the resolution.

The President reported the transfer of ownership of *CANCER RESEARCH* to the Association as given in the minutes of the meeting of the Board of Directors on April 15, 1949.

The proposal of the Board of Managers of *CANCER RESEARCH* regarding increasing the dues of active members and the approval of the Board of Directors were reported. After a spirited and general discussion it was voted that "The annual dues of active members be increased to ten dollars and that active membership carry with it a subscription to *CANCER RESEARCH*."

The President announced the results of the voting for new members of the Board of Directors and declared the new Directors to serve until 1952 to be Drs. J. C. Aub, E. V. Cowdry, E. A. Doisy, and W. U. Gardner.

The President also reported the action of the Board with respect to the order of names on the ballot and announced the decision of the Board regarding a member of the Board succeeding himself. These actions were approved.

The nominations for officers of the Association were read:

Joseph C. Aub, *President*
Jacob Furth, *Vice-President*
Charles W. Hooker, *Secretary-Treasurer*

The candidates were elected.

The conflicts of the meeting with other meetings of interest to members of the Association were pointed out. Dr. Frank B. Queen presented the resolution: "That the Board of Directors of the Association be requested to explore the possibility of coordinating the time and the place, and if not the place, the time, of other meetings with the Executive Boards of the American Association of Pathologists and Bacteriologists, the International Association of Medical Museums, and the American Society for Experimental Pathology; that it is the sense of the members of the Association that these meetings be so timed that the meetings of the several societies will not be mutually exclusive to their members." In the discussion the reason for the present custom of meeting in conjunction with the Federation and the belief that a majority of the Association's members are interested in the Federation's meeting were mentioned. Dr. M. J. Shear pointed out the difficulties experienced by employees of the Federal Government in getting numerous or closely spaced leaves of absence. When submitted to vote, Doctor Queen's resolution was adopted.

The desirability of having the abstracts printed prior to the meeting was pointed out, and the question was briefly discussed without decision.

The meeting was adjourned at 2:25 P.M.

CHARLES HUGGINS
President

CHARLES W. HOOKER
Secretary

MINUTES OF THE MEETING OF THE BOARD OF DIRECTORS HELD APRIL 17, 1949

The meeting was called to order at 1:15 P.M. in the Hotel Fort Shelby in Detroit, Michigan, following waiver of previous formal notice of the meeting signed

by all Directors present and constituting a quorum. Board members J. J. Bittner, A. M. Brues, E. V. Cowdry, E. A. Doisy, Jacob Furth, W. U. Gardner, C. W. Hooker, and Charles Huggins were present with the new Chairman of the Board, Dr. J. C. Aub, presiding.

Reading of the minutes of the last meeting was waived.

The problem of the relations of the Association with the press was discussed. Dr. Cowdry, who represented the Association in its relations with the press during the present meeting, reported that the Chairmen of the several scientific sessions had met with representatives of the press. It seemed agreed that public interest in the cancer problem and public support of cancer research more or less dictate cooperation with the press, and that the seriousness of the cancer problem makes it imperative that press reports not be misleading, however unintentionally. No ready solution was apparent, but it seemed agreed that if abstracts were available, science writers could come to the meeting better prepared to report papers presented. The advantages of prior publication of abstracts to members of the Association attending the meeting were also stressed. It was voted, "That abstracts of the papers on the program be published in the number of *CANCER RESEARCH* immediately preceding the meeting."

The problem of financing *CANCER RESEARCH* was again discussed, with particular attention to applications to various Foundations for support. It was voted, "That the Board of Directors seek any support that can be obtained without restrictions as to use."

The selection of a new Editor-in-Chief and the sacrifices an Editor must make were discussed. Several competent and desirable candidates were suggested, and means of making the post attractive were considered. The President was directed to proceed toward the selection of an Editor.

It was voted, "That the Executive Council take the necessary steps to transfer the funds of Cancer Research, Inc. to such place as they deem fit."

It was pointed out that the automatic subscription of members and the handling of non-member subscriptions by the publisher have rendered the post of Business Manager of *CANCER RESEARCH* unnecessary. It was therefore voted, "That the post of Business Manager of *CANCER RESEARCH* be discontinued; that the Business Manager be discharged with the thanks of the President and the Association; that the Secretary-Treasurer of the Association assume the residual duties of the Business Manager; and that the sum of $1200 be allocated for the office of Secretary-Treasurer." This sum represents $900 previously allocated by *CANCER RESEARCH* to the office of the Business Manager and $300 usually appropriated by the Association for the office of the Secretary-Treasurer.

The Chairman of the Board proposed the following as members of the standing committees of the Association:

Program Committee.—A. M. Brues, Chairman; C. P. Rhoads; H. P. Rusch.

Nominating Committee.—C. W. Hooker, Chairman; H. B. Andervont; E. V. Cowdry.

Membership Committee.—Jacob Furth, Chairman; W. L. Simpson; E. C. Reifenstein, Jr.

Committee on Cancer Research, Its Organization and Support.—Shields Warren, Chairman; G. M. Smith.

He also proposed the following Special Committees:

Committee on Press Relations.—E. V. Cowdry.

Committee on Amending By-Laws.—C. W. Hooker, Chairman; Jacob Furth.

The Board approved the proposed Committees.

The customary banking resolutions were adopted.

Publication of the minutes and scientific proceedings of the meeting were authorized, the costs of publication to be paid by the Association.

It was voted, "That publication of the By-Laws and list of members be deferred until after the next annual meeting." This action was prompted by the proposal that the By-Laws be revised and brought to date before the next meeting.

The meeting was adjourned at 2:00 P.M.

JOSEPH C. AUB
Chairman, Board of Directors

CHARLES W. HOOKER
Secretary